ADDRESSING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) AND OTHER TRANSBOUNDARY DISEASES FOR IMPROVED AQUATIC ANIMAL HEALTH IN SOUTHEAST ASIA

Rolando V. Pakingking Jr.
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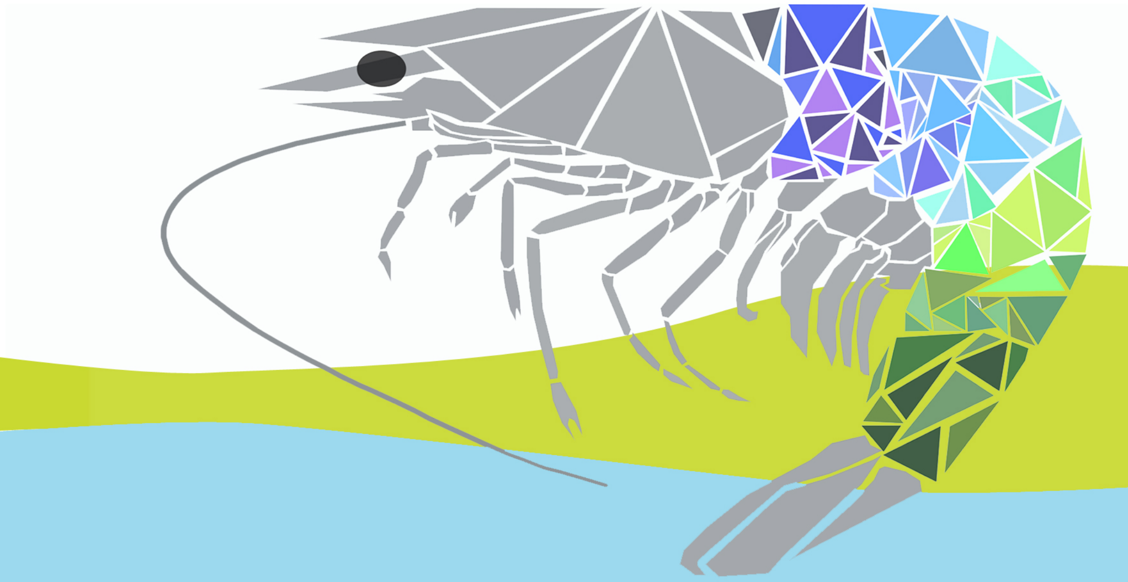
Editors



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Proceedings of the
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22-24 February 2016, Makati City, Philippines

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Association of Southeast Asian Nations

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Foreword

The need to provide adequate supply of nutritious food, especially cheap protein for the world population is becoming more urgent. Sufficiency and constant availability of the supply are the critical issues at stake. With productivity of the natural resources already stretched to its limit, feeding the fast growing population is becoming a big challenge and is also becoming more difficult to achieve. More innovations in science and technology are needed to increase productivity. For the fisheries sector, the twin approaches of proper resource management and responsible culture of aquatic organisms should be pursued together to make sure that our natural resources will continue to provide food on our tables in the future.

The ASEAN Member Countries depend heavily on the fisheries sector as a critical contributor to national and regional economic and social development. Countries in the region like Viet Nam, Thailand, Indonesia, Philippines and Myanmar are among the world's top producers of fishery products from aquaculture, and the other countries in the region are catching up. For aquaculture, however, to continue its important role of providing fish supply to our people, increasing further its productivity in a sustainable manner and without detrimental effect to the surrounding environment is necessary. This, however, is easier said than done since intensification of aquaculture operations to increase production oftentimes results in undesirable consequences.

Several factors affect the outcome of intensive aquaculture operations, and among them is the occurrence of diseases. The global aquaculture industry has had ups and downs, and the "downs" have been mostly caused by disease outbreaks that have resulted in mass mortalities and subsequent significant reduction in production. Take the case of the shrimp or the prawn industry. The collapse of the *Penaeus monodon* industry in Southeast Asia was associated with the outbreak of luminous bacteria and later with WSSV infection after culture systems have intensified. Until today, we still do not have effective cure for WSSV infection. Farmers in our region then shifted to growing *P. vannamei* to replace *P. monodon*. Production of *P. vannamei* was very high until another disease, EMS or AHPND hit the industry about 3-4 years ago. Shrimp farms in Thailand, Viet Nam and Malaysia were badly hit by this disease. Fortunately, other countries in the region are not yet affected by this disease. However, there are recent reports of AHPND in shrimp farms in the Philippines. We still do not know what triggers the proliferation of these bacteria in the culture systems. Shrimp farmers from EMS-affected countries have been employing various approaches to prevent the recurrence of this disease and have achieved some degree of success. Another disease that has recently become significant is hepatopancreatic microsporidiosis which has been detected in farms in Thailand, Viet Nam and Indonesia.

This publication presents the outputs of the *Regional Technical Consultation on EMS/AHPND and other Transboundary Diseases*. The information and experiences compiled in this publication will help us face disease problems or health management issues in the future. Farmers from countries who have not experienced these diseases will surely learn from those that have developed strategies or technologies for early disease detection, prevention and management.

We expect that the outputs of this Consultation will help guide the ASEAN Member States, regional organizations and other partners in the region in drawing up country-specific and regional policies that would avert impending epidemics that could affect the region's fishery sector.

Felix G. Ayson, D.Sc.
Former Chief, SEAFDEC/AQD

Message[1]

Distinguished guests and officials, ladies and gentlemen, good morning.

On behalf of the Government of Japan, it is a great honor and pleasure to speak before you all in today's ASEAN Regional Technical Consultation. Allow me to convey my sincere appreciation to the organizers for inviting me to this historical event.

Japan recognizes the need for regional efforts to focus on the aquaculture sector and other related aspects to support initiatives related to enhancing food security and safety. We emphasize the importance of the aquaculture industry particularly in the areas of job creation, contribution to gross domestic products, rural development and most importantly to food security in the Southeast Asian region. Moreover, we highlight the significance of aquatic animal health management and biosecurity in the sustainable development of aquaculture.

I congratulate the ASEAN Secretariat, SEAFDEC and Government of the Philippines for organizing this Regional Technical Consultation. Your proactive efforts are indeed laudable in protecting the aquaculture industry. Together, let us work towards sharing and exchanging our experiences and expertise on improving the aquaculture economy and contributing to food security and safety. In this regard, I feel very grateful and inspired that Japan's assistance in this endeavor enhances the strategic cooperation between and among the ASEAN member countries and its people.

Once again, I wish you all a successful consultation. Thank you very much.

Mr. Kenji Terada
First Secretary, Agriculture, Embassy of Japan in the Philippines

[1]Speech delivered by Mr. Kenji Terada at the opening ceremonies of the *Regional Technical Consultation on EMS/AHPND and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia*, *22-24 February 2016, Makati City, Philippines*

Message

As we are all aware, the aquaculture sub-sector in Southeast Asia particularly the shrimp industry has been shaken by the constant threat of viruses and outbreaks of transboundary diseases. The rapid growth of this sub-sector for the last two decades was threatened and impeded by this prevailing challenge. This is why we aim for an improved aquatic animal health management. As our way forward, we know that we must rely on our partnership or collaboration to come up with harmonized strategies and concrete actions.

In the Philippines, we had already proven that active collaboration among key players of the aquaculture industry propelled our resiliency in addressing the challenges brought about by diseases, environmental and other unpredictable production issues. The shared efforts of both the private sector and our government resulted to workable mitigating measures addressing broad negative impacts of emerging disease problems. This holds true when we were faced by two devastating shrimp diseases, the luminous bacteria infection in 1992 and the white spot syndrome virus infection in 1998. The integration of the Philippine Government's intervention and farmers' commitment in practicing prescribed farming innovations helped us identify the 'best-practices' in mitigating the effects of these diseases and enabled us to deal with new and emerging diseases. Aside from close collaboration, we believe that the key to disease-free aquaculture industry is the strict implementation of farming practices that do not harm our stocks/aquatic biota and the environment. With this in mind, and in line with our updated Comprehensive National Fisheries Industry Development Plan, one of our identified strategies towards improved yet sustainable aquaculture productivity in the next five years is the institutionalization of Good Aquaculture Practices.

We urge all ASEAN Member States and relevant stakeholders to continue exchanging information and latest updates, including the lessons learned. These information, if pooled together could serve as 'bricks' which would help us build an edifice of strong and reliable biosecurity measures in the Southeast Asian region. Placing these lessons before a broader perspective would bring forth improved and comprehensive strategies that can be used to counter EMS/AHPND and other transboundary diseases in Southeast Asia.

The publication of the Proceedings of the regional technical consultation is timely as this gives the readers, particularly those from the ASEAN Member States the opportunity to obtain new knowledge and better understanding on how to deal with EMS/AHPND and other transboundary diseases. The information, including the lessons learned if translated into good practices will help us achieve our common goal: to accelerate aquaculture growth by developing effective yet sustainable management mechanisms.

Our gratitude, as well, to the country representatives of the ASEAN Member States, the representatives from the private sector, and our resource persons.

Atty. Asis Perez
Former Undersecretary for Fisheries, Department of Agriculture and
Director, Bureau of Fisheries and Aquatic Resources

Message

We are all aware that during these past several years, the aquaculture industry of Southeast Asia had been confronted with continuing issues on transboundary diseases that hinder its sustainable development. This is especially true in shrimp culture which many countries in our region depend on, not only in terms of improving their economies, but also in enhancing the livelihood of fish farmers. Considering such a scenario, the Southeast Asian Fisheries Development Center (SEAFDEC) Council had considered the incidence of transboundary diseases in aquaculture as one of the priority areas that need to be addressed with much urgency.

Concomitant with the fast development of shrimp culture in the Southeast Asian region was the occurrence of various shrimp disease epidemics. These started with monodon baculovirus (MBV) followed by infectious hypodermal and hematopoietic necrosis virus (IHHNV), yellow head virus (YHV), and the taura syndrome virus (TSV). While the countries in the region were seriously working to establish effective approaches to prevent and control further incidence of such diseases, there came the white spot syndrome virus (WSSV) which was not only more destructive than the previous ones but spread rapidly in Southeast Asia. Nevertheless, through advancements in research, further serious occurrences of WSSV infection had been contained. Countries in the region continued exerting more efforts to transform the shrimp culture industry through the adoption of various measures including enforcement of biosecurity in shrimp hatcheries and ponds. The result was increased shrimp production and supply of cultured shrimps in the world market.

Recently however, we have learned of reports on the occurrence of acute hepatopancreatic necrosis disease (AHPND) which is more often referred to as early mortality syndrome (EMS) since mortality of infected shrimps occurs within 30 days after stocking the postlarvae. Such development has again threatened the sustainability of our shrimp culture industry and this time with more severity. Many countries have been working practically day and night to come up with remedies and measures to alleviate the impacts from such disease. In some Southeast Asian countries such as in Thailand, recent reports indicated the occurrence of another emerging disease in shrimp aquaculture such as hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (EHP). The list could go on, and there could be no end to the occurrence of diseases that would continue to threaten the sustainability of the region's aquaculture industry. It is therefore, an opportune time for countries in our region to work not independently but collaboratively to address the issues and come up with the most practical measures in order to stop these epidemics from occurring and recurring.

Considering the transboundary nature of these diseases that could easily spread from one place to another or even across the country or region, we should come up with the most appropriate surveillance measures. Putting in place such effective surveillance measures would alert the countries of any disease occurrence and adopt appropriate preventive and management measures in a timely manner and stop or avoid the transfer and spread of such diseases. However, in order to establish such measures, it has become necessary that a regional collaborative mechanism be developed. This regional meeting gave a good opportunity for countries and concerned agencies and institutions to work together to mitigate the impacts of any emerging transboundary diseases in the future. This also became an avenue where advancements in R&D as well as experiences in aquaculture diseases were exchanged and shared.

The ASEAN Network of Aquatic Animal Health Centres (ANAAHC), which is based in the Aquatic Animal Health Research Institute (AAHRI) of Thailand's Department of Fisheries, is an existing ASEAN mechanism which could be mobilized to supplement the national and regional efforts in addressing issues on aquatic animal diseases. ANAAHC could also serve as a focal point for enhancing the diagnostic and health certification capabilities among the ASEAN Member States. In view of this, ANAAHC could be tapped as a channel to facilitate the establishment of a regional collaboration.

On behalf of SEAFDEC, I would like to extend our utmost appreciation and gratitude to the ASEAN Secretariat and Japan-ASEAN Integration Fund for the financial support provided for the realization of this Consultation and publication of the Proceedings. Let us all bear in mind that our efforts could make the aquaculture industry of the Southeast Asian region sustainable and more food available in the plates of our people especially the small-scale fish farmers.

Kom Silapajarn, Ph.D.
SEAFDEC Secretary-General and
Chief of SEAFDEC Training Department

Acknowledgements

We gratefully acknowledge the Government of Japan through the Japan-ASEAN Integrated Fund, administered by the ASEAN Secretariat, for the main financial support. We also thank the Philippine Bureau of Fisheries and Aquatic Resources for co-organizing the meeting and for the in-kind contributions. Special thanks are also due to all meeting participants, particularly the Member Country representatives and the Panel of Experts for their invaluable contributions to the meeting.

The editors would like to thank the members of SEAFDEC/AQD's Publications Review Committee for reviewing the Proceedings prior to its publication. Thanks are also due to the Development Communication Section of the Training and Information Division of SEAFDEC/AQD, led by Mr. Rex Dianala, for copy-editing and layout. And lastly, to Mr. Isidro Tendencia for the design and concept of the cover.

Photo of participants

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Background

Aquaculture in Southeast Asia has grown rapidly especially during the last two decades. The most serious problems faced by the aquaculture sector are diseases spread and introduced through movements of hatchery produced stocks, new species for aquaculture, and development and enhancement of the ornamental fish trade. A number of infectious diseases have emerged threatening the sustainability of aquaculture in the region. The occurrence of aquatic diseases has not only led to low production but has also threatened food security and raised food safety and environmental concerns. The early mortality syndrome (EMS) or currently known as acute hepatopancreatic necrosis disease (AHPND) is one of the diseases that resulted in heavy mortalities of cultured shrimp in several Asian countries, where one million people depend on shrimp aquaculture for their livelihoods. EMS outbreaks resulted in significant drops in shrimp production in Malaysia, Thailand and Viet Nam.

During the 2012 and 2013 meetings of the Southeast Asian Fisheries Development Center (SEAFDEC) Program Committee, member country representatives expressed concern about the outbreaks of EMS/AHPND and other transboundary diseases in the region and noted the need for concerted regional effort to address this. In response, the SEAFDEC Council, during its meeting in April 2014, suggested that aquatic animal health management, including control and prevention of transboundary aquatic animal diseases, be included in the formulation of future programs of SEAFDEC and its partners in the region.

Recognizing the pressing need for sustained regional efforts to address disease problems in farmed aquatic animals, particularly on shrimps, SEAFDEC Aquaculture Department and Government of the Philippines (Department of Agriculture-Bureau of Fisheries and Aquatic Resources), through the financial support from the Japan-ASEAN Integration Fund, convened the *Regional Technical Consultation on EMS/AHPND and other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia* from 22 to 24 February 2016 in Makati City, Philippines. Over 60 delegates representing the technical experts, the ASEAN Member States and Japan, regional and international organizations and private sector attended the Consultation. The participants assessed the status of AHPND and other transboundary aquatic animal diseases in ASEAN Member States, identified gaps, priority areas for research and development collaboration and formulated regional policy recommendations which are outlined in the suceeding page.

Policy Recommendations

Issues/Gaps	Regional Policy Recommendations
Legislative and policy frameworks	• Develop National Strategy and Policy Frameworks • Member countries to harmonize legislation and regulations related to aquatic animal health management, particularly the legislation for transboundary movement of live aquatic animals
Strategy for prevention, control, and biosecurity	• Promote compliance with good aquaculture practices to maintain optimal environmental conditions during the culture period • Establish effective prevention system for EMS/AHPND and other diseases based on R&D results • Develop and implement the Guidelines on Health Management and Good Practices to prevent EMS/AHPND and other transboundary diseases • Strictly implement the reporting system to relevant authorities and/or Competent Authority at country, regional and international levels (Early warning system, Monitoring system, Information for the regular report, Annual report) • Develop emergency preparedness and contingency plans (should be the responsibility of Competent Authority) • Private and public sectors to ensure fund availability and consider this as joint endeavor
Rapid and reliable detection of EMS/AHPND	• Diagnostic methods: should follow the World Organization for Animal Health (OIE) guidelines • Develop tool kits • Ensure availability and capacity of laboratory services, either public or private
Research and development program at regional and national levels	• Use of live feeds for broodstock (specifically polychaetes) – potential carriers of pathogen • Effects of inbreeding/genetic erosion on susceptibility to AHPND • Toxin plasmid transfer to other *Vibrio* species and possibly other bacterial pathogens • Vertical transmission, risk factors, mixed infection • Use of greenwater technology, probiotics, new disease prevention and control strategies
Cooperation among relevant stakeholders	• Strengthen cooperation arrangements among ASEAN Member States (AMS), international/regional organizations such as OIE, Food and Agriculture Organization of the United Nations (FAO), Network of Aquaculture Centres in Asia and Pacific (NACA), SEAFDEC and ASEAN Network of Aquatic Animal Health Centres (ANAAHC)
Capacity building program	• This should also include technology transfer from AMS to another AMS
Awareness building	• Enhance awareness of farmers and relevant stakeholders on R&D developments in transboundary diseases (especially on management and control)

Latest Research on Acute Hepatopancreatic Necrosis Disease (AHPND) of Penaeid Shrimps

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ABSTRACT

Acute hepatopancreatic necrosis disease (AHPND) is caused by unique strains of *Vibrio parahaemolyticus* (VP_{AHPND}) and *V. harveyi* that have transferrable plasmid carrying the virulent PirAB-like toxin genes. The genomes of VP_{AHPND} strains and *V. harveyi* from Thailand and Viet Nam, respectively, have been characterized by our group. The genome of VP_{AHPND} strains from Mexico, Viet Nam, and China have also been studied by other groups. We have developed a conventional polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) methods for the detection of AHPND using a primer set that targets the PirAB-like toxin genes of VP_{AHPND}. We have characterized the toxin genes of VP_{AHPND} strains and also constructed a recombinant plasmid (broad host range) carrying PirAB-like toxin genes. Non-VP_{AHPND} strain N7 which does not carry the plasmid and strain FP11 which is carrying a plasmid not coding for the toxin genes were transformed with the plasmid carrying PirAB-like toxin genes. As a result, the transformed N7 and FP11 strains became virulent and killed whiteleg shrimp (*Penaeus vannamei*) similar to or at par with the virulence of VP_{AHPND} strain. We then fed the whiteleg shrimp with commercial feed containing the formalin-killed VP_{AHPND} strain. After 2 days of feeding, all of the whiteleg shrimp died. These results clearly indicate that the PirAB-like toxin is the virulence factor of VP_{AHPND}.

We have been investigating the virulence mechanism of the PirAB-like toxin produced by VP_{AHPND} strains. First, we calculated the copy number of plasmid encoding the PirAB-like toxin genes of several VP_{AHPND} strains. The copy number of the plasmid varied, ranging from 1 to 36 copies. Interestingly, VP_{AHPND} strains carrying low copy number of plasmid were more virulent than VP_{AHPND} strains carrying high copy number of the plasmid. These results imply that the copy number of toxin genes is not an important factor responsible for the degree of virulence of the VP_{AHPND} strains. We are also studying other factors associated with the virulence of PirAB-like toxin. Likewise, we are developing prevention methods against AHPND including the use of formalin-killed cell vaccine, IgY additive in feed, and nano-bubble treatment of rearing water. This paper summarizes the current R&D on the disease.

Introduction

Acute hepatopancreatic necrosis disease (AHPND), formerly known as early mortality syndrome (EMS), is a devastating disease that has been implicated in mass mortality of cultivated penaeid shrimps in China (2009), Viet Nam (2010), Malaysia (2011), Thailand (2012), Mexico (2014), and recently in the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; Dabu *et al.*, 2015; dela Peña *et al.*, 2015). AHPND induces necrosis in the hepatopancreas of the infected shrimp causing them to become lethargic and anorexic. The disease was first referred to as early mortality syndrome (EMS) because it was typified by shrimp mortality occurring within 30 days after stocking shrimp postlarvae (PL) in grow-out ponds (Lightner *et al.*, 2012). However, there was confusion regarding the usage of the term EMS as mortalities during the early phase of shrimp cultivation could also be attributed to other etiologies, hence, the more precise name AHPND was adapted after the group of D.V. Lightner discovered in 2013 that unique strains of *V. parahaemolyticus* (VP_{AHPND}) colonizing the stomach of shrimp produced toxin responsible for the sloughing of hepatopancreatic tubule epithelial cells of the hepatopancreas (Tran *et al.*, 2013). By employing an infection bioassay, i.e. through an immersion challenge using *V. parahaemolyticus* isolated from ponds with AHPND outbreak, 100% mortality was obtained in naive shrimp; as the dead shrimp exhibited sloughing of the tubule epithelial cells of the hepatopancreas, thereby satisfying Koch's postulates (Tran *et al.*, 2013). Moreover, they also documented that cell-free broth cultures of VP_{AHPND} could induce the massive sloughing of the hepatopancreatic tubule cells even in the absence of bacterial cells (Tran *et al.*, 2013). Fortunately, all VP_{AHPND} isolates tested did not possess the pathogenicity island (human pathogen markers *tdh* and *trh*) associated with human infections (Nishibuchi *et al.*, 1985; Nishibuchi *et al.*, 1989).

Previous studies on the genome sequences of *V. parahaemolyticus* strains that caused and did not cause AHPND were established to identify the virulence genes that might be involved in the pathogenicity of VP_{AHPND} strains to cultured penaeids (Kondo *et al.*, 2014; Yang *et al.*, 2014; Gomez-Gil *et al.*, 2014). These studies revealed that VP_{AHPND} strains possess a unique 69-kbp plasmid carrying the suspected genes homologous to PirAB toxin genes that encodes for the *Photorhabdus* insect-related (Pir) toxins (Lee *et al.*, 2015), indicating that the PirAB-like toxins are the virulence factors of VP_{AHPND} strains.

Characterization of the causative agent of AHPND and its virulence to penaeid shrimps

AHPND is caused by unique strains of *V. parahaemolyticus* (VP_{AHPND}) and *V. harveyi* that have a transferrable virulent plasmid carrying the PirAB-like toxin genes (Figure 1). However, non-VP_{AHPND} strains also possess the plasmid (Figure 2). To ascertain that shrimps are indeed infected with AHPND, the target region for detection employing the polymerase chain reaction (PCR) method should be the toxin region. The genomes of VP_{AHPND} strains from Thailand, Mexico, and China, as well as *V. harveyi* from Viet Nam and *V. owensii* from China have been studied by several research groups (Kondo *et al.*, 2014, 2015; Yang *et al.*, 2014, Gomez-Gil, *et al.*, 2014, Liu *et al.*, 2015). The findings indicate that spreading of the plasmid coding for the PirAB toxin genes among several bacterial species in the shrimp pond is feasible. This finding further indicates that in worst case scenarios, plasmid transfer from VP_{AHPND} to normal bacterial microbiota in the shrimp pond may inadvertently occur and could result in unwarranted outbreaks of AHPND among pond-cultivated shrimps (Figure 3).

We have developed the conventional PCR method using a primer set that targets the PirA-like toxin gene of *V. parahaemolyticus* (Tinwongger *et al.*, 2014). At present, the

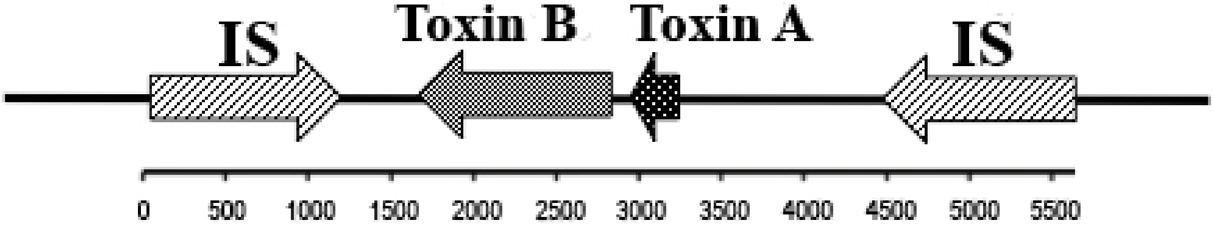

Figure 1. Structure of the toxin genes region of VP_{AHPND}.

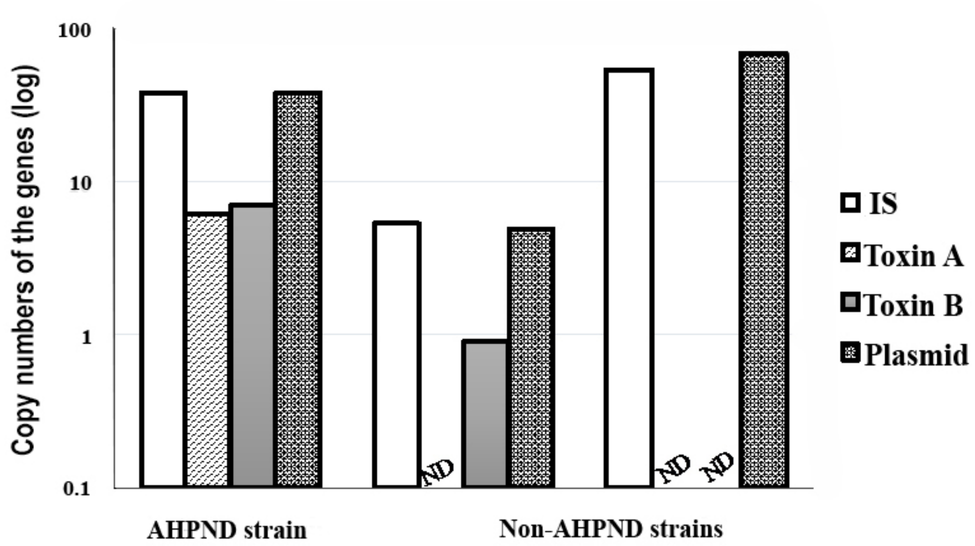

Figure 2. Relative copy number of toxin genes in VP_{AHPND} and non-VP_{AHPND} strains compared to the ToxR gene which is a single copy gene on the genome. ND: Not detected.

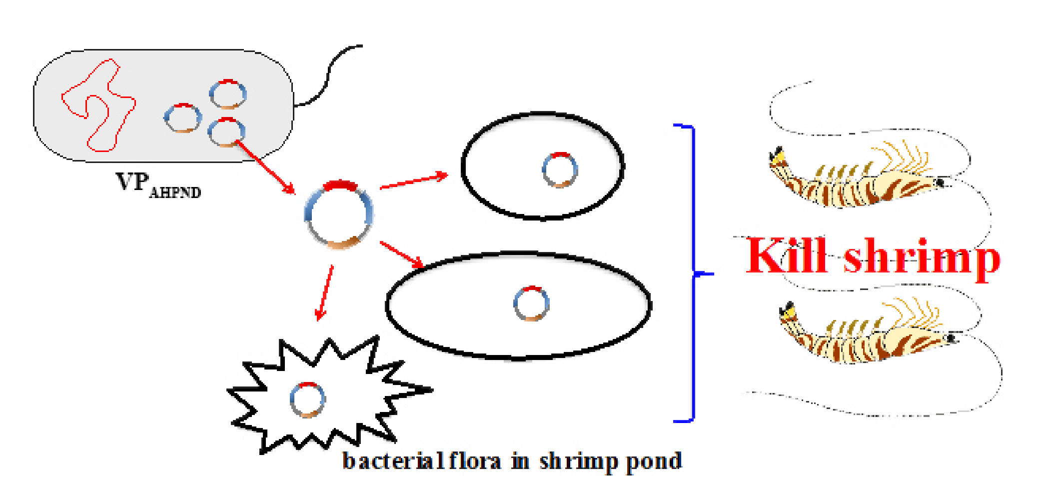

Figure 3. Hypothetical illustration of a worst case scenario of AHPND outbreak in cultured shrimps through inadvertent transfer of plasmid coding for the PirAB toxin genes from VP_{AHPND} to other species of bacterial flora in the shrimp pond.

accuracy of our diagnostic methods for AHPND is 100%. We have also developed a loop-mediated isothermal amplification (LAMP) method for the detection of VP_{AHPND} strains (Koiwai *et al.*, 2016).

We have also confirmed the virulence of PirA-like and PirB-like toxins by using transformant of non-VP_{AHPND} carrying the toxin genes in the broad host range plasmid (Tinwongger *et al.*, 2016, unpublished data). The transformant of non-VP_{AHPND} strain showed virulence to whiteleg (*P. vannamei*) and kuruma (*Marsupenaeus japonicus*) shrimps. The virulence level of this transformed strain was similar to that of VP_{AHPND} strain. In addition, we studied the virulence of a natural mutant from highly virulent VP_{AHPND} strain. Because a major part of the toxin genes of this mutant strain was naturally deleted during the course of culture in nutrient broth, our results clearly show that this strain lost its virulence to whiteleg shrimp (Tinwongger *et al.*, 2016, unpublished data). Moreover, these results indicate that the toxins are the virulence factors of VP_{AHPND} strain.

The region encoding the toxin genes is composed of approximately 6 kbp and exhibits terminal inverted repeats of about 1.2 kbp (Tinwongger *et al.*, 2016, unpublished data). The repeats encode insertion sequence (IS). The IS encodes transposase and is identical to other reported strains of *V. parahaemolyticus* (Kamruzzaman and Nishibuchi, 2008). The non-virulent strains carrying the plasmid completely lack the toxin region, but possess an IS. Interestingly, we found that the virulent strains also possess the region lacking toxin genes but have a single IS. These results suggest that the IS might have transposase activity which is involved in deletion and/or insertion of the toxin genes (Figure 4). Importantly, these results denote that several colonies will be necessary for PCR diagnosis of AHPND especially when using bacteria grown on agar plates.

We compared the virulence of five different VP_{AHPND} strains to shrimp (Tinwongger *et al.*, 2016, unpublished data). We also studied the copy number of plasmids in these five VP_{AHPND} strains. Two of these strains have a low copy number of the plasmid and toxin genes. The other three strains have more than 30 copies of the plasmid and toxin genes. Interestingly, VP_{AHPND} strains carrying low copy number of plasmid were more virulent than VP_{AHPND} strains carrying high copy number of the plasmid. These results imply that the copy number of toxin genes is not an important factor responsible for the degree of virulence of the VP_{AHPND} strains. We further studied the secretion of toxin of these five VP_{AHPND} strains. The most virulent strain secreted the highest amount of toxin compared to other VP_{AHPND} strains, suggesting that virulence of VP_{AHPND} strains corresponded to the amount of secreted toxin.

Development of prevention methods against AHPND

With regard to the development of pragmatic, effective, and economically-sound prevention and control methods against AHPND, we have been investigating the potential use of VP_{AHPND} formalin-killed cells (FKC) as vaccine immunogen, IgY additive in feed, nano-bubble technology, phage therapy, and the isolation and characterization of toxin receptors in the host.

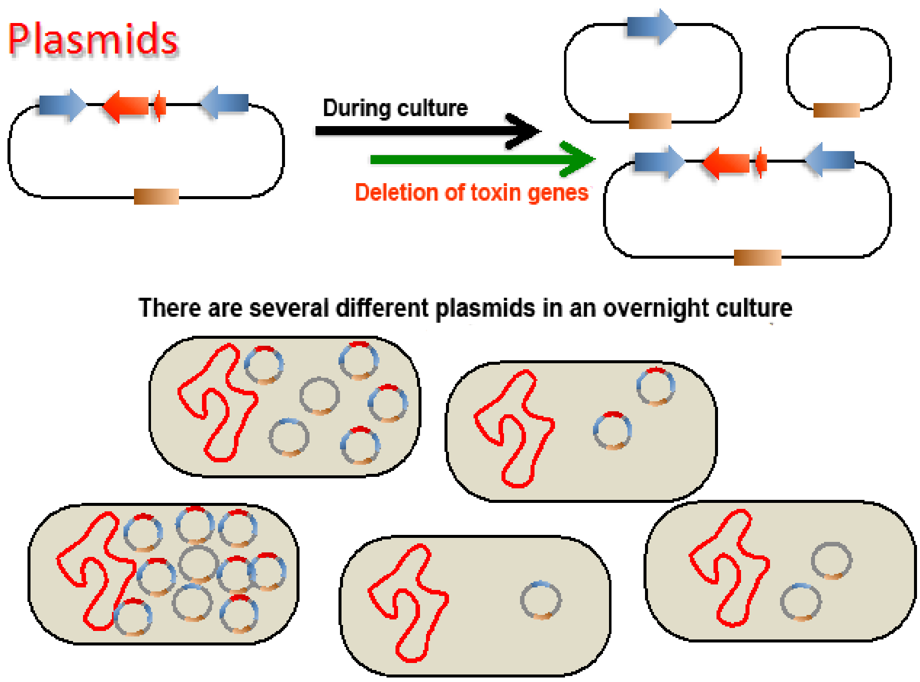

Figure 4. Insertion sequence-mediated production of several types of AHPND plasmid during culture of VP_{AHPND}.

Formalin-killed cell vaccine trials

Shrimps do not have a known adaptive immune system. However, there have been several reports showing the efficacy of vaccines targeted for infectious diseases of shrimp. Based on these reports, it was suggested that shrimp may have an unknown and unique adaptive immune system which may be completely different from the vertebrate's adaptive immune system.

We tested the efficacy of the formalin-killed cell (FKC)-VP_{AHPND} vaccine using whiteleg shrimps weighing 5 to 7 g. VP_{AHPND} was cultured in a broth at 30°C for 18 hours. After incubation, cultured VP_{AHPND} was treated with formalin for 24 hours at 4°C. When FKC-VP_{AHPND} was fed to shrimp, many shrimps died within few days post-feeding. This result suggests that the VP_{AHPND} toxin was stable in formalin and heating at 60°C. We conducted bath vaccinations using 3 different formalin-killed cells (FKC)-VP_{AHPND} strains (TUMSAT-N1, A1 and FP1) as vaccine immunogen. We added 200 mL of the FKC-VP_{AHPND} to 20 L of sea water. Then shrimp were immersed in FKC-VP_{AHPND}-seawater mixture for 2 hrs. After the bath vaccination, shrimp were transferred to another tank for periodic observation. Representative result of the VP_{AHPND} challenge test conducted at post-bath vaccination with the FKC-VP_{AHPND} is shown in Figure 5. One of the FKC-VP_{AHPND} vaccines, i.e. TUMSAT-FP1, conferred good protection in shrimp experimentally challenged with VP_{AHPND}. This result indicates that not all VP_{AHPND} strains have protective antigens against the homologous VP_{AHPND} strains. In another experiment, we used small shrimps with a mean body weight of 0.8 g. FKC-VP_{AHPND} vaccine did not confer protection to small shrimp experimentally challenged with VP_{AHPND}, indicating that the immune system of small shrimp may not have developed yet.

IgY: Immunoglobulin in egg yolk

IgY is a specific antibody of birds especially present in their eggs. When a female chicken was immunized with recombinant VP_{AHPND} toxins, it produced eggs containing high titers of IgY specific for VP_{AHPND} toxins. Furthermore, we used the egg yolk extract as feed additive and successfully demonstrated its efficacy in conferring protection in shrimp experimentally challenged with VP_{AHPND} strain. In addition,

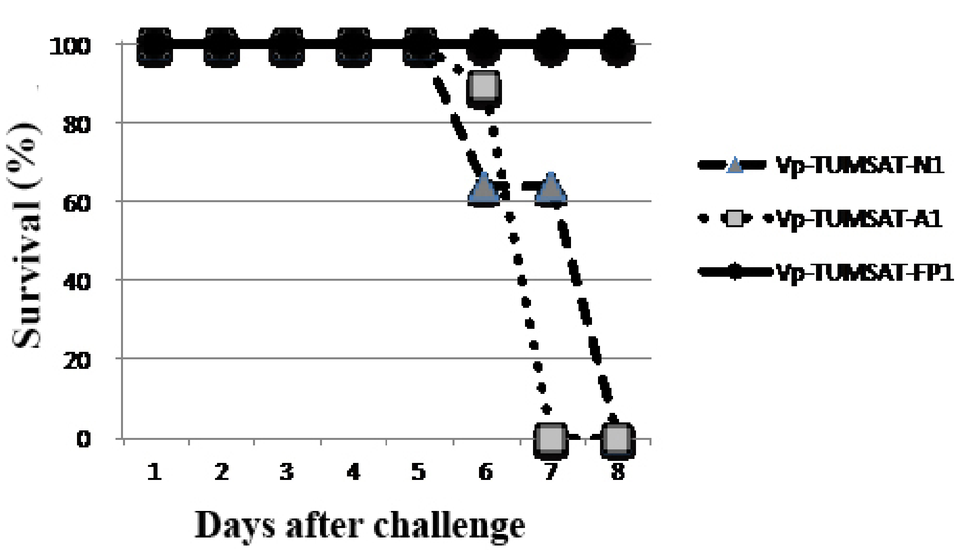

Figure 5. Survival rates (%) of bath-vaccinated (formalin-killed VP_{AHPND} strains) whiteleg shrimp (*Penaeus vannamei*) challenged with a virulent VP_{AHPND} strain.

our data clearly indicate the potential of incorporating IgY in feed as a practical strategy to prevent AHPND outbreaks in shrimp most particularly during the early stage (first 20-30 days) of culture.

Receptors of toxins

Proteins with high homology to VP_{AHPND} toxins in other bacteria have been well characterized. These toxins bind to some receptors of host insects causing some damages to the host's digestive system. However, several reports revealed that toxin-resistant individuals exist in nature. Such toxin-resistant individuals have a mutated receptor for the specified toxins. It is therefore seemingly evident that in nature, there are certain individual shrimps that are resistant to the VP_{AHPND} toxin. To find an AHPND-resistant individual and/or family, it is prudent to identify their receptors for VP_{AHPND} toxin in shrimp. We are currently conducting some experiments focusing on the receptors for VP_{AHPND} toxin in penaeid shrimps by using next generation sequencing and immunological methods using anti-toxin antibody.

Nano-bubble water

Treatment with ozone-nano-bubble water could reduce mortality of shrimps infected with VP_{AHPND} strain. In addition, ozone-nano-bubble water treatment of shrimp could confer protection against WSSV infection. However, we still have to conduct more experiments to thoroughly elucidate the efficacy of nano-bubble water technology for the prevention and/or treatment of microbial infections in penaeid shrimps. Likewise, its practical application in grow-out cultivation ponds needs to be looked into.

Way forward

Our completed and ongoing studies aim to generate information geared at preventing and controlling AHPND in cultivated shrimps. Experiments focusing on the virulence mechanisms of the VP_{AHPND} toxin and effects on the immune responses of shrimp are being carried out. Experiments aimed at elucidating the receptors for VP_{AHPND} toxin in the host's cells are likewise being conducted. Notably, we have observed that a low percentage of shrimp could survive after exposure to VP_{AHPND} toxin, indicating that these surviving shrimp might be resistant to AHPND. However, more data need to be generated to substantiate this speculation.

Hepatopancreatic microsporidiosis (HPM) caused by the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) has also been recently recognized as an economically important parasitic disease of cultured shrimps. However, information on HPM-EHP is scarce. We have already started analyzing the genome of EHP. We believe that the data that will be generated from our ongoing EHP genome analysis will be pivotal in the establishment of accurate diagnostic methods needed in the formulation of effective, pragmatic, and economically-sound approaches against HPM in cultured penaeids.

Acknowledgements

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OIE Initiatives on Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Aquatic Animal Diseases in Asia

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Abstract

The World Organization for Animal Health (OIE) is an intergovernmental organization established in 1924 responsible for improving animal health and welfare worldwide to facilitate safe international trade of animals and animal products while avoiding unnecessary impediments to trade. OIE, as a reference organization of the World Trade Organization (WTO), works to set and update its international standards (OIE Codes and Manuals) regularly through transparent and democratic procedures. The Aquatic Code defines an OIE list of notifiable aquatic animal diseases according to the criteria for listing, which comprise consequences, spread and diagnosis. To be listed, a disease should meet the criteria of each characteristic defined in the Aquatic Code. The acute hepatopancreatic necrosis disease (AHPND) has been officially included in the OIE-listed diseases since May 2015 and officially enforced since 1 January 2016. To fulfill its overall vision which can be summarized by its slogan *Protect animals and Preserve our Future*, the OIE Regional Representation in Tokyo, Japan and Sub-Regional Representation in Bangkok, Thailand, are working in concert to provide regionally adapted services to OIE Members so that surveillance and control of animal diseases in the region may be strengthened.

Introduction

The World Organization for Animal Health (formerly the Office International des Epizooties or OIE) is an intergovernmental organization established in 1924 responsible for improving animal health and welfare worldwide (OIE, 2016a). In the current trend of globalization, animal health measures have increasing importance to facilitate a safe international trade of animals and animal products while avoiding unnecessary impediments to trade. In light of this, the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) encourages the members of the World Trade Organization (WTO) to base their sanitary measures on international standards, guidelines and recommendations, where they exist (OIE, 2016g). The objective of listing is to support Member Countries' efforts to prevent the transboundary spread of important diseases of aquatic animals through transparent and consistent reporting. The corresponding disease-specific chapters in the Aquatic Code provide standards for safe international trade in aquatic animals and their products (OIE, 2016b).

The OIE Aquatic Animal Health Code (the Aquatic Code) sets out standards for the improvement of aquatic animal health and welfare of farmed fish worldwide, and for safe international trade in aquatic animals (amphibians, crustaceans, fish and molluscs) and their products. The health measures in the Aquatic Code should be used by the Competent Authorities of importing and exporting countries for early detection, reporting and control of agents pathogenic to aquatic animals and to prevent their transfer via international trade in aquatic animals and their products, while avoiding unjustified sanitary barriers to trade (OIE, 2016b).

OIE code and manual

The OIE is recognized by the WTO as a reference organization for standards relating to animal health and zoonoses including aquatic animal diseases (OIE, 2016e). The OIE publishes 2 codes (Terrestrial and Aquatic) and 2 manuals (Terrestrial and Aquatic) as the principal references for WTO members.

The Terrestrial Animal Health Code (Terrestrial Code) and the Aquatic Animal Health Code (Aquatic Code) respectively aim to assure the sanitary safety of international trade in terrestrial animals and aquatic animals, and their products (OIE, 2016g).

The Terrestrial Code was first published in 1968 and the Aquatic Code was introduced to the public in 1995. The codes traditionally addressed animal health and zoonoses, however, in recent years, they have expanded to cover animal welfare, animal production, food safety, consistent with the expanded mandate of the OIE which is to improve animal health worldwide (OIE, 2016j). The same adoption applies more or less to the Aquatic Code as well.

The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual) and the Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual) provide a harmonized approach to disease diagnosis by describing internationally agreed laboratory diagnostic techniques. These manuals were published in 1989 and in 1995, respectively (OIE, 2016g).

The OIE regularly updates its international standards as new scientific information comes to light, following its established transparent and democratic procedures. The development of these standards and recommendations is the result of the ongoing work by the OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) (OIE, 2016g). This Commission, founded in 1960 and comprises six elected members, meets twice yearly to address its work programme (OIE, 2016c). The Commission is elected by the International Committee for a three-year term. The current President of the Commission is Dr. Ingo Ernst from Australia (Department of Agriculture and Water Resources) with Professor Mohamed Shariff Bin Mohamed Din from Malaysia (University of Putra Malaysia) as its member (OIE, 2016d).

This Commission compiles information on diseases of fish, molluscs and crustaceans and on methods used to control these diseases and draws upon the expertise of internationally renowned specialists to prepare draft texts for new articles of the Aquatic Code and the Aquatic Manual and to revise existing articles. The Commission also organizes scientific meetings on diverse topics of importance to aquaculture (OIE, 2016c).

The only pathway for adoption of a standard is via approval of the World Assembly of Delegates meeting in May each year at the OIE General Assembly (OIE, 2016g). The views of OIE national delegates are routinely sought through the twice yearly circulation of new or revised texts. Member countries are strongly encouraged to get involved more actively in the OIE standard setting process.

OIE listed diseases

The Aquatic Code defines the OIE list of notifiable aquatic animal diseases according to the criteria for listing, which comprise consequences, spread and diagnosis. To be listed, a disease should meet the criteria of each characteristic defined in the Aquatic Code. The list is reviewed on a regular basis and in case of modifications adopted by the World Assembly of Delegates at its annual General Session, the new list comes into force on 1 January of the following year. For year 2016, the list includes 28 aquatic animal diseases, out of which, 9 diseases are specifically for shrimps and/or prawn (Table 1) (OIE, 2016h).

Diseases proposed for listing should meet the relevant criteria as set out in (a) consequences, (b) spread, and (c) diagnosis. Such proposals should be accompanied by a case definition for the disease under consideration (OIE, 2016b).

Table 1. OIE listed diseases of crustaceans

No.	Disease	Susceptible Species[1]
1	Acute hepatopancreatic necrosis disease[2]	• Giant tiger prawn (*Penaeus monodon*) • Whiteleg shrimp/Pacific white shrimp (*P. vannamei*)
2	Crayfish plague (*Aphanomyces astaci*)	• All species of crayfish in all three crayfish families (*Cambaridae*, *Astacidae* and *Parastacidae*)
3	Infection with yellow head virus	• Giant tiger prawn (*P. monodon*) • Whiteleg shrimp/Pacific white shrimp (*P. vannamei*) • Blue shrimp (*P. stylirostris*) • Dagger blade grass shrimp (*Palaemonetes pugio*) • Jinga shrimp (*Metapenaeus affinis*)
4	Infectious hypodermal and haematopoietic necrosis	• Giant tiger prawn (*P. monodon*) • Whiteleg shrimp/Pacific white shrimp (*P. vannamei*) • Blue shrimp (*P. stylirostris*)
5	Infectious myonecrosis	• Whiteleg shrimp/Pacific white shrimp (*P. vannamei*)
6	Necrotising hepatopancreatitis	• Whiteleg shrimp/Pacific white shrimp (*P. vannamei*) • Blue shrimp (*P. stylirostris*) • Northern white shrimp (*P. setiferus*) • Northern brown shrimp (*P. aztecus*)
7	Taura syndrome	• Whiteleg shrimp/Pacific white shrimp (*P. vannamei*) • Blue shrimp (*P. stylirostris*) • Northern white shrimp (*P. setiferus*) • Southern white shrimp (*P. schmitti*) • Greasyback prawn (*M. ensis*) • Giant tiger prawn (*P. monodon*)
8	White spot disease	• All decapod (order *Decapoda*) crustaceans from marine, brackish and freshwater sources.
9	White tail disease	• Giant freshwater prawn (*Macrobrachium rosenbergii*)

[1] For the purposes on the Aquatic Code chapter, the terms shrimp and prawn are used interchangeably (OIE, 2016b)
[2] From the Chapter Acute hepatopancreatic necrosis disease (AHPND); still on drafting process (COMMISSION, 2016)

Given the inclusion of acute hepatopancreatic necrosis disease (AHPND) in Chapter 1.3. Diseases listed by the OIE, at the May 2015 OIE General Session, the Aquatic Animals Commission developed a new draft chapter on AHPND for inclusion in the Aquatic Code in October 2015 (COMMISSION, 2015). In February 2016, revised text for the Aquatic Code and the Aquatic Manual including new draft chapter for AHPND have been circulated to Member countries for their comments (COMMISSION, 2016). All the comments will be considered by the Commission at its September 2016 meeting (OIE, 2016f).

The revised text included several technical information for AHPND. Based on the new draft chapter for AHPND (COMMISSION, 2016) or the purposes of the Aquatic Code, AHPND means infection with strains of the bacteria *Vibrio parahaemolyticus* (VP_{AHPND}) and *V. harveyi* that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB. The susceptible species are whiteleg shrimp (*P. vannamei*) and giant tiger prawn (*P. monodon*). Competent Authorities should not require any conditions related to AHPND, regardless of the AHPND status of the exporting country, zone or compartment, when authorizing the importation or transit of the following aquatic animal products from whiteleg (*P. vannamei*) and giant tiger prawn (*P. monodon*):

- heat sterilized hermetically sealed crustacean products (i.e., heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
- cooked crustacean products that have been subjected to heat treatment at 100°C for at least 3 minutes (or any time/temperature equivalent which has been demonstrated to inactivate VP_{AHPND};
- pasteurized crustacean products that have been subjected to heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent which has been demonstrated to inactivate VP_{AHPND};
- crustacean oil; and
- crustacean meal

The comprehensive technical report on AHPND presented at the *Meeting of the OIE Aquatic Animal Health Standards Commission* in Paris last 15-19 February 2016 will be thoroughly described in the new draft chapter for AHPND.

Disease Reporting System

Each OIE Member Country undertakes to report the animal diseases that it detects on its territory. The OIE then disseminates the information to other countries, which can take the necessary preventive action. The OIE created and manages the World Animal Health Information System (WAHIS) which is coupled with WAHIS interface, providing information on 118 diseases listed for 2016. WAHIS Interface provides public access to all data regarding OIE-Listed diseases, which improves the transparency, efficiency and speed with which animal health information is disseminated throughout the world (OIE, 2016k).

The OIE Regional Representation for Asia and the Pacific (RRAP) is collaborating with the Network of Aquaculture Centres in Asia-Pacific (NACA) on the Quarterly Aquatic Animal Disease (QAAD) Report since 1998, which covers not only OIE-listed diseases but also non-OIE listed diseases of regional importance. AHPND was first included in the QAAD as a non-OIE listed disease, while OIE later agreed to add AHPND to the OIE listed diseases in May 2015, coming into force from 2016.

This example demonstrates the importance, validity and usefulness of reporting and sharing the disease information particularly that of regional concerns through OIE-NACA QAAD Report. On the other hand, past experience shows that there had been certain inconsistencies in provided data between WAHIS and QAAD, including delayed or failed reporting by member countries. It is crucial to advocate improving immediate and accurate disease reporting from all member countries with the cooperation of all stakeholders.

Activities of OIE RRAP to support member countries

The *Second Regional Work Plan Framework 2016-2020* of the OIE Regional Commission for Asia and the Pacific, adopted by the OIE Regional Commission in September 2015, highlights the increasing importance of Aquatic Animal Health in this region and encourages members as well as OIE to strengthen the activities on promoting Aquatic Animal Health (OIE Regional Commission for Asia, 2015). The RRAP is conducting regional capacity building activities, such as the *Seminar for the OIE National Focal Points*, latest one held in January 2015 in Ho Chi Minh City, Viet Nam, the *Regional Workshop on Safe International Trade in Aquatic Animals and Aquatic Animal Products* held in July 2015, in Nagaoka City, Japan.

Regardless of whether veterinarians are involved in the Aquatic Animal Health Services (AAHS), it is clear that the general principles for quality would be similar to those that apply to Veterinary Services. For example, appropriate legislation and good governance are required to support AAHS in complying with OIE requirements, including for disease detection, reporting and control. The application of the Performance of Veterinary Services (PVS) Tool to the evaluation of AAHS commenced in 2009 when the OIE undertook a pilot mission in Viet Nam.

The OIE RRAP continues to support member countries through these activities to facilitate networking between OIE Delegates and OIE National Focal Points and eventually strengthen the disease surveillance, early detection and rapid response at a national level (OIE, 2016i).

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Acute Hepatopancreatic Necrosis Disease (AHPND) of Penaeid Shrimps: Global Perspective

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Abstract

The Organization for Economic Cooperation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations Agricultural Outlook 2015-2024 reported that fisheries production worldwide is projected to expand by 19% between the 2012-14 base period and 2024, to reach 191 million metric tons (MT) and the main driver of this increase will be aquaculture, which is expected to reach 96 million MT by 2024, 38% higher than the base period (average 2012-14) level. Among the 7 key uncertainties that affect gains in productivity, the potential of animal disease outbreaks to affect aquaculture production and subsequently domestic and international markets are once again highlighted, although for the first time in this outlook. Another milestone document, the “Blue frontiers: managing the environmental costs of aquaculture” identified a number of fish health issues, including increased risk of the spread of pathogens and diseases with intensification, through increased movement of aquatic animals, inter-regional trade and introduction of new species and new strains, and through the use of trash fish or live feed; concerns on residues and development of drug resistant pathogens brought about by the abuse on the use antimicrobials and other veterinary drugs; limited availability of vaccines; environmental stressors that compromise the immune system; difficulties faced by developing countries in implementing international standards; and the need for legislation, enforcement and capacity building. The issues identified then and now are almost the same.

Addressing animal health issues in aquaculture is very challenging because the sector is highly complex (with a wide range of diversity in terms of species, systems, practices and environment, each presenting different risks), its fluid environment, and the transboundary nature where fish is considered as one of the most traded commodity, aquatic animals require more attention in order to monitor their health: they are not visible except in tank holding conditions; they live in a complex and dynamic environment and feed consumption and mortalities are hidden under water.

This paper looks at the status of a newly emerging disease of cultured shrimp, acute hepatopancreatic necrosis disease (AHPND), which has been recognized as the most important non-viral disease threat to cultured shrimp. In particular, this paper presents the highlights of the International Technical Seminar/Workshop: “EMS/AHPND: Government, Scientist and Farmer Responses” held from 22–24 June 2015 in Panama City, Panama, which was organized under the auspices of an FAO inter-regional project TCP/INT/3502: Reducing and Managing the Risks of AHPND of Cultured Shrimp, being participated by 11 countries, namely: Colombia, Ecuador, Guatemala, Honduras, Mexico, Panama and Peru from Latin America and the Caribbean (LAC) region and India, Iran, the Philippines and Sri Lanka from the Asian region. The Panama EMS/AHPND June 2015 event aimed to provide a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. More than 100 stakeholders from 21 countries representing the government, academe and producer sectors participated in the event. The highlights contain the latest available information at that time (June 2015) about AHPND including the current state of knowledge about the causative agent, the host and geographical distribution, detection methods, risk factors, management and actions of regional and international organizations.

Introduction

The Food and Agriculture Organization (FAO) Global Aquaculture Production statistics database has registered a total of 575 aquatic species and species groups grown in freshwater, seawater and brackishwater. In terms of value, the total food fish value was USD 150 million while crustaceans were valued at USD 31 million (FAO, 2015). Crustaceans are an important source of aquatic food protein. Production (as food and ornamental) and trade are extremely important for developing countries. It provides both economic development and empowerment in terms of contribution to gross domestic product (GDP), consumption, employment, catch value and exports. The crustacean sector generates high value export products which enables producers to buy lower value products in the world market - thus a positive contribution to food security in both producing and exporting countries (Bondad-Reantaso *et al.*, 2012). However, the crustacean sector is facing huge challenges due to disease outbreaks.

The OECD-FAO Agricultural Outlook is a collaborative effort of the Organization for Economic Co-operation and Development and FAO. It brings together the commodity, policy and country expertise of both organizations and input from collaborating member countries to provide an annual assessment of prospects for the coming decade of national, regional and global agricultural commodity markets. The OECD-FAO Agricultural Outlook 2015 for the first time had included fish as one of the commodities assessed. The outlook reported that by 2015, aquaculture is projected to surpass capture fisheries as the most important source of fish for human consumption, and by 2020 should represent about 45% of total fishery production (including non-food uses). Compared to the 2008-2010 period, average capture fish prices are expected to be about 20% higher by 2020 in nominal terms compared with a 50% increase for aquaculture species. Fisheries production worldwide is projected to expand by 19% between the 2012-14 base period and 2024, to reach 191 million MT and the main driver of this increase will be aquaculture, which is expected to reach 96 million MT by 2024, 38% higher than the base period (average 2012-2014) level. The outlook identified a number of key uncertainties affecting productivity gains. These include the following: natural productivity of fish stocks and ecosystem; occurrence of El Niño; fish meal and fish oil outlook; trade policies, and in particular bilateral trade agreements, remain an important factor influencing the dynamics of the world fish markets; availability and accessibility to land and water; financial resources; improvement in technology; and feeds, etc. The outlook reported that in addition, animal disease outbreaks have shown the potential to affect aquaculture production and subsequently domestic and international markets depending on the size and the species involved (OECD/Food and Agriculture Organization of the United Nations, 2015).

Hall *et al.* (2011), in a milestone publication of WorldFish Center and Conservation International Blue Frontiers[1] made a comprehensive assessment of how the global aquaculture industry uses natural resources and its impacts on the environment. They have identified a number of fish health issues such as:

- increasing risk of the spread of pathogens and diseases with intensification, farms getting larger and more concentrated;
- increasing movement of aquatic animals, inter-regional trade and introduction of new species and new strains increases the risk;
- use of trash fish as another risk factor in pathogen transfer;
- abuse on the use of antimicrobials and other veterinary drugs, concerns on residues and development of drug resistant pathogens;
- availability of vaccines limited for a few species and against few diseases;
- environmental stressors compromising the immune system;
- international standards more widely applied by developed countries; developing countries facing difficulties; and
- legislation, implementation and capacity building needed

[1]http://www.conservation.org/publications/documents/BlueFrontiers_aquaculture_report.pdf

Transboundary aquatic animal diseases or TAADS have the following characteristics: highly contagious/transmissible (infectious!); has the potential for very rapid spread irrespective of national borders (no passport!); cause serious socio-economic and possibly health consequences (high risk and high impact!); one of the negative impacts of trade globalization (important pathway!). The World Organization for Animal Health (OIE) lists about 27 aquatic pathogens/diseases which fit established criteria for listed diseases in terms of consequence, spread and diagnosis (important to trade!) (Bondad-Reantaso *et al.*, 2005).

Diseases in aquaculture can be infectious or non-infectious. Infectious diseases can be categorized into three. The first group are diseases that are important to trade (OIE list of diseases), governed by international standards, with a set of criteria to be met to be included in the list, pathogens/diseases of important traded species (e.g. finfish, crustaceans, mollusks), mandatory reporting/notification is required during an outbreak. The second group are diseases that are consistently affecting aquaculture species at the hatchery, nursery and grow-out levels, and may be caused by bacteria, parasites, fungi, and viruses. In addition, the third group are emerging diseases; these can be known diseases which have spread into new geographical areas or affected new susceptible species and diseases of unknown aetiology.

FAO TCP/INT/3502 Reducing and Managing the Risks of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp

The FAO inter-regional project, TCP/INT/3502 'Reducing and Managing the Risks of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp', is being participated by 11 countries, namely: Colombia, Ecuador, Guatemala, Honduras, Mexico, Panama and Peru from Latin America and the Caribbean (LAC) region and India, Iran, Philippines and Sri Lanka from the Asian region. One of the major outcomes expected from this project is enhanced knowledge and strengthened capacities for dealing with AHPND in the Asian and LAC regions. The conduct of an international technical seminar involving resource experts from the government, academe and producer sectors is one of the mechanisms that will contribute to achieving the above expected outcome. The International Technical Seminar/Workshop: "EMS/AHPND: Government, Scientist and Farmer Responses" was held from 22–24 June 2015 in Panama City, Panama. This technical seminar was implemented under the auspices of the project TCP/INT/3502 and was jointly organized with OIRSA, the Regional International Organization for Plant Protection and Animal Health. The objective of the Panama EMS/AHPND June 2015 event was to provide a platform to improve the understanding of the disease through the lens of governments, scientists and producers and collectively generate practical management and control measures. More than 100 stakeholders from 21 countries representing the government, academe and producer sectors participated in the event. The highlights contain the latest available information at that time (June 2015) about AHPND including the current state of knowledge about the causative agent, the host and geographical distribution, detection methods, risk factors, management and actions of regional and international organizations. The information presented below was based on the 21 technical presentations by resource experts representing the government, academe and producer sectors. Drs. J.R. Arthur and M. Reantaso led the writing of the summary and highlights with comments and contributions received from Drs. C. Lavilla-Pitogo, I. Karunasagar, and V. Alday-Sanz.

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

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

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

The Network of Aquaculture Centres in Asia-Pacific (NACA) Quarterly Aquatic Animal Disease (QAAD) reporting system[2] includes AHPND in the NACA regional list of disease. A request for the inclusion of AHPND in the List of Notifiable Diseases of the World Organization for Animal Health's (OIE) was submitted in 2014, but the OIE Aquatic Animal Health Standards Commission (AAHSC) did not endorse the listing during its February 2014 meeting since AHPND did not meet all the criteria for disease listing. However, in March 2015, the AAHSC endorsed the listing of AHPND and a final decision to this effect was made during the OIE General Session held in May 2015. Reporting of AHPND to OIE commenced in January 2016.

Current state of knowledge about AHPND

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

Current host and geographic distribution

AHPND first appeared in the People's Republic of China (around 2009 and was called Covert Mortality Disease), and has since been recorded from Viet Nam (2011), Malaysia (2011), Thailand (2012), Mexico (2013 from the scientific literature) and the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; Dabu *et al.*, 2015; dela Peña *et al.*, 2015). It is suspected to be present in, but unreported from other countries in both Asia and Latin America and the Caribbean (LAC). The disease infects mainly whiteleg shrimp (*Penaeus vannamei*), but has also been reported from giant tiger prawn (*P. mondon*) and fleshy prawn (*P. chinensis*).

Current status of detection methods to diagnose AHPND

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

[2]http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report

tubule epithelium in the absence of any clear evidence of a causative agent.

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

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

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

[3]www.enaca.org

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

Risk factors

The most important risk factors for the international spread of AHPND are:

- movement of live shrimp from a geographic region where AHPND is prevalent to an unaffected region for aquaculture (AHPND is thought to have been transmitted to Mexico from Asia by this route).
- the importation of live animals (e.g. polychaetes, clams) as feeds for shrimp broodstock (polychaetes imported from P.R. China may have been the major route for introduction of AHPND to Thailand).

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

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

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

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

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

Most cases of VP_{AHPND} have shown co-infection with other shrimp pathogens, for example, monodon baculovirus (MBV), white spot syndrome virus (WSSV), hepatopancreatic parvovirus (HPV), *Enterocytozoon hepatopenaei* (EHP) and unidentified gregarine-like entities.

Disease management

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

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

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

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

Actions of International and Regional Organizations

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

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

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

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

(ii) the First Inter-regional Workshop on EMS/AHPND Risk Management and Risk Reduction Strategies at National and Regional Levels, 25–27 June 2015. A second interregional seminar/workshop under this TCP will be held in Asia in 2016 (Bangkok EMS/AHPND June 2016).

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Regional Response on AHPND and Other Emerging Shrimp Diseases in the Asia-Pacific

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Abstract

Transboundary aquatic animal diseases are among the major concerns for establishing biosecurity measures and strengthening of aquatic animal health (AAH) management capacity (including emergency preparedness) in the region. In aquaculture, biosecurity and AAH management entails protection of fish or shellfish from infectious agents (viral, bacterial, fungal or parasitic) as well as prevention of disease spread from one area to another. Several transboundary aquatic animal diseases have swept the region over the past 25 years causing massive economic and social losses. These include spread and outbreaks of epizootic ulcerative syndrome (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (e.g. white spot disease [WSD], infectious haematopoietic necrosis [IHHN]). The spread of these transboundary diseases clearly demonstrates the vulnerability of the aquaculture industry to disease emergence where impacts have been aggravated by the lack of effective preparedness and response when diseases emerge.

Recently, outbreaks of acute hepatopancreatic necrosis disease (AHPND), popularly known as early mortality syndrome (EMS), among cultured shrimps were reported in China and Viet Nam (2010), Malaysia (2011), Thailand (2012), Mexico (2013) and the Philippines (2014). There have been reports of its spread in South American countries but limited report is available in this regard. This disease caused significant losses in the production of *Penaeus monodon* and *P. vannamei* in the affected countries. NACA's regional response to this disease during its initial outbreak in Viet Nam, Thailand and Malaysia signified that improved control on transboundary diseases and emergency preparedness are still needed in the region. In collaboration with international organizations (OIE, FAO), NACA has implemented awareness programs, efficient information dissemination, and emergency regional expert consultation to address this disease problem. OIE and FAO also deployed experts to assess the disease and identify the pathogen involved. All of these efforts, together with subsequent studies on prevention and disease management, have paved the way in preventing further spread of this disease to other shrimp-producing countries so far. However, the risk is still very high that this disease will spread, as transboundary movement of live shrimps within and outside the region is inevitable. In addition, other emerging diseases are now affecting production of major cultured shrimps in the region. These include hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) with confirmed reports from China, Viet Nam, Thailand, Malaysia and Indonesia (unconfirmed reports from India), and viral covert mortality disease (VCMD) which was reported to be affecting cultured shrimps in China.

By and large, outbreaks of damaging aquatic animal diseases are likely to continue and the potential consequences are likely to increase with the expansion (intensification) of aquaculture systems and introduction of new species for culture. Consequently, the risks associated with emerging and transboundary diseases are shared – shared water bodies and epidemiological links through trade (especially live movement) – thus, a collaborative approach in dealing with these diseases is therefore warranted and necessary.

Introduction

Aquaculture is one of the important sectors in the economies of the ASEAN Member States (AMS). However, majority of aquaculture farms are small-scale and most often lack the necessary facilities to comply with or not well informed of the product standards imposed by concerned authorities, especially for international trade. Although some AMS (e.g. Thailand, Philippines, Malaysia, Indonesia and Viet Nam) have made significant progress in disease surveillance and aquatic animal health certification for biosecurity purposes, which is required for exported aquaculture products (both live and processed), other less-developed countries in the region have made very limited progress in this regard.

It is, therefore, necessary for these countries to develop and implement national as well as regional biosecurity frameworks to minimize the impacts of aquatic animal diseases, prevent the introduction and spread of infectious diseases, and to produce high quality and safe aquaculture products. The continuous globalization in the aquaculture trade has increased the potential of disease introduction and spread to new areas. Important aquatic animal diseases have been reported to spread among major aquaculture-producing and -exporting countries, and can usually cause serious economic losses, as well as some social and ecological consequences. The movement of live aquatic biota (plants and animals), their products and the water they are in has the potential to transfer pathogens from one country or region to another where the pathogens may not currently exist. In shrimps as example, most major disease outbreaks were associated with the movement of live animals (broodstock, nauplii and postlarvae) when the patterns of disease spread were analyzed. Transboundary diseases are among the major concerns for establishing biosecurity measures and strengthening of aquatic animal health management capacity in the region. Many aquatic animal diseases, once established, are often difficult to treat or to eliminate.

Several transboundary aquatic animal diseases have swept the region over the past 25 years causing massive economic and social losses. These include spread and outbreaks of Infection with *Aphanomyces invadans* (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (white spot disease [WSD], white tail disease [WTD], yellow head disease [YHD]) (Rogers *et al.*, 2011). More recently, infectious myonecrosis (IMN) and acute hepatopancreatic necrosis disease (AHPND) are seriously affecting shrimp aquaculture in Indonesia (IMN; Senapin *et al.*, 2007), Malaysia, Philippines, Thailand and Viet Nam (AHPND; Flegel, 2012; Leaño and Mohan, 2012; Dabu *et al.*, 2015). The spread of these transboundary aquatic animal diseases clearly demonstrates the vulnerability of the aquaculture industry, as well as the wild fish populations, to disease emergence where impacts have been exacerbated by the lack of effective preparedness and response when diseases emerge. It is also necessary that aquafarmers, especially small-scale, be properly informed and educated on the current market standards being imposed by most importing countries, so that they can produce aquaculture products that can be considered safe for trading and consumption based on these standards on aquatic animal health and food safety.

AHPND and its spread

AHPND is a new disease causing unusually heavy mortality in cultured shrimps at approximately 30-45 days of culture. It was first reported in 2009 (officially in 2010) in China as a novel disease of unknown aetiology in shrimps and was initially named early mortality syndrome (EMS). The disease has spread to Viet Nam (2010), Malaysia (2011), Thailand (2012) (Flegel, 2012; Leaño and Mohan, 2012; Joshi *et al.*, 2014), Mexico (2013) (Nunan *et al.*, 2014; Gomez-Gil *et al.*, 2014), and the Philippines (2014) (NACA-FAO 2015; Dabu *et al.*, 2015; dela Peña *et al.*, 2015).

Clinical signs observed include slow growth, corkscrew swimming, loose shells, as well as pale coloration. Affected shrimp also consistently show an abnormal hepatopancreas (HP) (shrunken and discolored). The key diagnostic features needed for confirmation is the medial sloughing off of HP cells as seen in histological sections of the affected shrimp's HP. Other histopathological features of AHPND in both *P. monodon* and *P. vannamei*, which appear to be limited to the HP, are the following (Prachumwat *et al.*, 2012; Tran *et al.*, 2013):

- Lack of mitotic activity in generative E cells of the HP;
- Dysfunction of central hepatopancreatic B, F and R cells;
- Prominent karyomegaly and massive sloughing of central HP tubule epithelial cells;
- Terminal stages including massive intertubular hemocytic aggregation followed by secondary bacterial infections.

The disease is caused by a highly virulent strain of *Vibrio parahaemolyticus* (Tran *et al.*, 2013), carrying plasmids containing the genes that code for the toxins pirA and pirB (Han *et al.*, 2015; Lee *et al.*, 2015). Recently, genome sequencing has identified a non-*V. parahaemolyticus* associated with diseased shrimp in Viet Nam that also contain the same toxin plasmid, and indicated that it was *Vibrio harveyi* (Kondo *et al.*, 2015). A strain of *V. owensii* causing serious AHPND in shrimp, was also found to contain the plasmid similar to that of *V. parahaemolyticus*, indicating that the plasmid plays an important role in shrimp AHPND (Liu *et al.*, 2015).

NACA's regional response on EMS/ AHPNS/AHPND

In April 2011, Viet Nam, which has been suffering from the devastating effect of AHPND (then known as EMS) for more than a year, officially sought assistance from regional and international organizations including the Network of Aquaculture Centres in Asia-Pacific (NACA), the World Organization for Animal Health (OIE) and the Food and Agriculture Organisation of the United Nations (FAO). OIE and FAO immediately sent emergency missions to Viet Nam, to investigate and make a quick assessment of the disease problem. NACA fully coordinated and collaborated with both OIE and FAO in the implementation of subsequent projects to solve the disease problem. In November 2011, the NACA's Asia Regional Advisory Group on Aquatic Animal Health discussed the issue during its 10th Meeting held in Mangalore, India. The disease was referred to by Prof. Timothy Flegel as acute hepatopancreatic degenerative necrosis syndrome (AHDNS). Recognizing the importance/threat of AHDNS to the shrimp industry in the region, the Advisory Group gave the following recommendations to NACA during the meeting (NACA, 2011):

- Make a request to Dr. Donald V. Lightner (Arizona State University) to prepare the case definition of the disease. NACA will then prepare the corresponding Disease Card for circulation to NACA member countries and uploading in the NACA website.
- Encourage member governments to report any occurrence/outbreak of AHDNS through immediate reporting and/or the QAAD Reporting System.

On the other hand, initial findings of the different emergency missions in Viet Nam suggested an infectious etiology based on how the disease has spread. Other agents, however, were not discounted including environmental causes (e.g. toxins, pesticides, etc.). In May 2012, a formal Circular and a Disease Advisory were released by NACA and widely disseminated to Competent Authorities (CA), partner institutions and other relevant organizations in the Asia-Pacific region and the world. The Advisory was also published in key publications for wider dissemination of information regarding this emerging shrimp disease problem (Leaño and Mohan, 2012). NACA also made all the necessary efforts to look for donors which can possibly fund an emergency regional consultation to discuss the current status of the disease.

The Australian Department of Agriculture, Fishery and Forestry (now known as Australian Department of Agriculture) provided NACA the necessary funds to organize the emergency regional consultation, which was held in August 2012 in Bangkok, Thailand. The primary objectives of the consultation were to:

- bring together global experts, national participants representing CAs and lead research institutions, regional and international organizations and the private sector;
- facilitate networking and information sharing for better understanding and dealing with EMS;
- document the current state of knowledge on EMS and lessons learned in dealing with disease emergencies at national/ regional levels; and
- agree on a regional action plan for dealing with future aquatic disease emergencies in the region.

The consultation was attended by 17 global shrimp health experts and 65 representatives from NACA member countries, lead research institutions in the region, other regional and international organizations, national universities and private sectors. The proceedings of the consultation was published in a Technical Report which includes the latest updates and review of status of EMS, case definition at animal and pond level, R&D directions, and recommendations for preventive measures at both national and regional levels (NACA, 2012a).

In September 2012, in collaboration with Prof. Donald V. Lightner and Prof. Timothy Flegel, NACA released the Disease Card for AHPNS (NACA, 2012b), which contains relevant updates and information about the syndrome (prior to the identification of the causative pathogen) which was widely disseminated to relevant authorities and institutions in the region. This was for the purpose of harmonizing research effort and supporting disease surveillance and disease outbreak investigations. The NACA AG, during its 11th meeting held in Bangkok, Thailand, also recommended the inclusion of AHPNS in the Quarterly Aquatic Animal Disease (QAAD) Reporting in the Asia-Pacific, commencing in the January-March 2013 reporting period (NACA-FAO, 2013). This was mainly for the purpose of gaining more information and updates on the occurrence and spread of AHPNS in the region.

After the first Regional Consultation, many reports have circulated among the stakeholders in the shrimp industry on the identification of causative agent of the disease, without any scientific bases to prove such claims. Thus, in March 2013, NACA released a second Disease Advisory to address the many circulating false and baseless speculations on the effects, spread and causative agent of AHPNS (NACA, 2013a). After the release of the second Advisory, the team of Dr. Lightner finally identified the causative agent of the disease as a virulent strain of *V. parahaemolyticus*. This was reported in a paper in the journal Diseases of Aquatic Organisms (Tran *et al.*, 2013). With this development and the recommendations made from the FAO TCP in Viet Nam (FAO, 2013), the NACA AG officially renamed the disease to AHPND and recommended to revise the Disease Card to include the identification of the pathogen, bioassay procedures, rapid diagnostic test, and other recent information about the disease (NACA, 2013b). The name in the QAAD list was also changed accordingly, effective the January-March 2014 reporting period.

Currently, the disease is being reported (through QAAD reporting) from China, Philippines, Thailand and Viet Nam. Malaysia's recent reports are questionable, as they have reported the non-occurrence of the disease since the 3rd quarter of 2014, although there are still reports of outbreaks in the country.

On diagnostics, several AHPND detection methods including PCR-based protocols have been developed. The use of AP1 to AP4 primers for PCR-based detection was first announced at the NACA website starting 2013. Primers AP3 and AP4 target the AHPND toxin genes pirA and pirB (Sirikharin *et al.*, 2015; Dangtip *et al.*, 2015), and these primers are released for free, together with positive control plasmids, courtesy of Prof. Timothy Flegel's research team. Confirmatory diagnosis of the disease is still based on histopathology of HP and infection experiment/bioassay (if possible).

AHPND is now included in the OIE list of reportable diseases after its endorsement in May 2015 during the OIE General Session. The Chapters for both the OIE Aquatic Animal Health Code and Manual are being prepared for endorsement during the GS in May 2016. Official reporting to OIE commenced in January 2016.

As there is no control measure presently available for AHPND, several preventive measures are recommended one of which is the use of clean broodstock and PLs which has been made possible by screening using the different PCR detection methods that have been developed. Additionally, the use of healthy broodstock and production of healthy and strong PLs were recommended to prevent infection, while improved environmental and feeding management as well as efficient biosecurity measures will prevent outbreaks and spread of the disease.

Other emerging shrimp diseases

Heptopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei *(HPM-EHP)*

Hepatopancreatic microsporidiosis was first seen way back in 2001 in the HP of *P. modonon* (Chayaburakul *et al.*, 2004) and the pathogen identified (*E. hepatopenaei*; EHP) in 2009 (Tourtip *et al.*, 2009). It was discovered in slow growing shrimp but was not statistically associated with slow growth at that time (Sritunyalucksana *et al.*, 2014) Recently, EHP was found in shrimps with white feces syndrome, but was confirmed to be not the causative agent of the disease (Tangprasittipap *et al.*, 2013). However, it was also reported by Tangprasittipap *et al.* (2013) that EHP could infect exotic *P. vannamei* imported for cultivation in Asia and that it could be transmitted directly from shrimp to shrimp via the oral route.

HPM-EHP shows no clear gross signs for diagnosis, but when severe growth retardation is observed among cultured shrimps, then it can be suspected as a possible cause. To confirm diagnosis, histology, wet mount microscopic observation and/or molecular methods are required (NACA, 2015a). The disease causes retarded growth when copies above 103/ng total HP DNA is observed (Liu *et al.*, 2016). No significant mortality can be observed among shrimps affected by the disease.

HPM-EHP is widespread in China, Malaysia, Thailand and Viet Nam, and probably India (Sritunyalucksana *et al.*, 2014). In response to the growing concerns on the effect and spread of this disease, a Disease Card was prepared and published (NACA, 2015a), while the NACA AG agreed and recommended its listing in QAAD Reporting (NACA, 2015b), starting at the first quarter of 2016.

Viral covert mortality disease (VCMD)

VCMD is caused by covert mortality nodavirus (CMNV), which has caused serious losses among cultured shrimps in China since 2009 (Zhang *et al.*, 2014). Clinical signs include hepatopancreatic atrophy and necrosis, empty stomach and guts, soft shell, slow growth, and whitish muscle in the abdomen (Zhang, 2014; Huang, 2012). Affected shrimps usually die at the pond bottom, and mortality can be observed daily during 30-80 days of culture, peaking during 60-80 days of culture with cumulative mortality of up to 80% (Zhang *et al.*, 2014). The increase in mortality is usually accompanied by increase of nitrite-nitrogen and high temperature above 28°C.

Diagnosis of the disease include histopathological observation (Zhang *et al.*, 2014) of affected shrimps showing separated HP tubules with haemocytic infiltration, nuclear karyomegaly and eosinophilic intracellular inclusions in the tubular epithelium of HP. In some cases, swollen nucleoli could be found in hepatopancreocytes. The muscle fibres composing the whitish muscle lesion have muscle fragmentation tending towards coagulative muscular lysis and myonecrosis. Multifocal myonecrosis in the striated muscle is accompanied by haemocytic infiltration and karyopyknosis. Other diagnostic methods include transmission electron microscopy (Zhang *et al.*, 2014), fluorescence *in situ* hybridization, nested RT-PCR, RT-LAMP (Zhang *et al.*, in press), and by highly sensitive detection kits that have been developed.

Surveillance of the disease using molecular diagnostic methods revealed that the disease is widespread in shrimp culture provinces of China. In 2014, about 18% of the >300 samples collected from 10 coastal provinces in China were positive for CMNV. The virus was detected not only in *P. vannamei*, but also in *P. chinensis*, *P. japonicus*, *Macrobrachium rosenbergii* and the swimming crab *Portunus trituberculatus* (Huang, 2015). Positives in samples of different life stages, including nauplii, postlarva, juveniles, and broodstock, were also detected. Samples positive for the virus from other countries in Asia and Americas were also detected.

As agreed and recommended by the NACA AG, a disease card for VCMD is now being prepared. The disease card will contain information about the disease including clinical signs, different methodologies for diagnosis, as well as list of experts. It will then be considered for inclusion in the QAAD Reporting to encourage surveillance and reporting.

Conclusion

The initial outbreaks of AHPND in major shrimp-producing countries in the ASEAN has proven the importance of information dissemination and awareness programs for both affected and non-affected countries, which has prevented the further spread of the disease. So far, the disease did not spread to other shrimp-producing countries, as relevant biosecurity measures were put into place to prevent entry of the disease (e.g. Indonesia). Dealing with disease emergencies like AHPND will require emergency funds to immediately assess the status and impacts of the disease. FAO and OIE emergency missions to Viet Nam were undertaken a few months after Viet Nam sought assistance. However, regional action was only initiated after more than one year (through an emergency regional consultation organized by NACA) due to lack of funds to undertake such important activity. Moreover, coordinated and collaborative works are necessary in order to establish the nature of the disease (e.g. epidemiology, identification of the pathogen, diagnostics), as well as in the formulation of preventive measures and disease management strategies.

Outbreaks of important and emerging aquatic animal diseases will likely continue with the continuous expansion or intensification of aquaculture systems and introduction of new species for culture. The risks associated with these emerging and transboundary diseases are shared, as countries in the region have shared water bodies and epidemiological links through trade and transboundary movement of live aquatic animals. Therefore, a collaborative approach in dealing with these diseases is warranted and highly necessary for better aquatic animal health management at both national and regional levels.

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Current Status of Shrimp Farming and Diseases in Cambodia

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Abstract

The farming of penaeid shrimps in Cambodia began in 1989 and has significantly expanded since 1991. Shrimp cultivation has been carried out in the four coastal provinces, i.e. Kampot, Kep, Preah Sihanouk Ville, and Koh Kong. Black tiger shrimp (*Penaeus monodon*) and whiteleg shrimp (*P. vannamei*) are the main species being cultured extensively and intensively in brackishwater ponds in Kampot, Kep, and Preah Sihanouk Ville, and Koh Kong, respectively. Extensive shrimp ponds were constructed close to the mangrove areas with some containing mangroves within the pond and stocking density ranging from 5,000 to 20,000 postlarvae/ha. However, the productivity remains low at >100 kg/ ha/ year. On the contrary, intensive culture has a stocking density ranging from 300,000 to 500,000 postlarvae/ha. While high cost of investment for farm establishment, pond construction and farm operation are required, productions of newly established farms have reached 7 to 8 metric tons (MT) /ha per crop.

The occurrence of white spot disease, monodon baculovirus disease, and yellow head disease was first reported in 1999 among cultured *P. monodon* in Koh Kong province causing a number of farmers to stop the intensive cultivation of black tiger shrimp. To date, only a small proportion of shrimp farmers have ventured into extensive shrimp farming with approximately 10 ha of shrimp areas currently in operation. To mitigate the negative impacts of shrimp diseases and promote the expansion of the shrimp industry in Cambodia, development of a national reporting system for aquatic animal diseases; capacity building for detection, monitoring and disease surveillance; creation of National Guidelines On Good Shrimp Aquaculture Practices; establishment of sub-research centers and concomitant funding support for marine aquaculture development and extension services; establishment of local shrimp hatcheries and provision of hands-on trainings for farmers; and strengthening collaborations among provincial officers, researchers and farmers' network should be accordingly instituted.

Introduction

Cambodia is located in Southeast Asia between latitudes 10^0 and 15^0 N and longitudes 102^0 and 108^0 E, and has a mainland area of 181,035 km^2 extending approximately 580 km from east to west and 450 km from north to south. It has a total population of over 15 million people in 2015 with a population growth rate of 2.4% per annum which is reported to be the highest in Asia. The south and south-west of the country constitute a 443 km long coast at the Gulf of Thailand, characterized by sizable mangrove marshes, peninsulas, sandy beaches, and headlands and bays. There are four coastal provinces, located along this coastline namely Kep, Kampot, Preah Sihanouk Ville, and Koh Kong provinces. There are 69 islands, including the four large islands of Koh Kong, Koh Rong, Koh Sanlem, and Koh Thmey, and a number of small islands which are located near shore. The other three main islands namely Koh Tang, Koh Pring and Koh Polowaii are located further offshore. The Exclusive Economic Zone (EEZ) covers approximately 55,000 km and is relatively shallow with a depth of about 50 m. The fisheries sector in Cambodia plays an important role in its national economy as it employs approximately 4 million or 30% of its population contributing 10 to 12% of the Gross Domestic Product. Importance is also high in the aspect of food security as Cambodians obtain 70% of animal protein from fisheries products. Increasing fisheries production through marine aquaculture has been the major goal in the next few years as production from marine capture fisheries remains broadly flat in recent years. The increase in marine aquaculture production is also expected to result in the suppression and reduction of excessive pressure on the coastal fisheries resources and will therefore facilitate the resource recovery. It has been recognized that development of aquaculture in Cambodia should be achieved in conjunction with the conservation of coastal fisheries environment. The priority areas identified in The Strategic Planning Framework for Fisheries (SPFF 2010 – 2019) adopted by the Fisheries Administration includes improving livelihoods for poor population through sustainable utilization of fisheries resources, reduction of post-harvest losses in captured fisheries products, revisions of policy, plans and legislations pertaining to fisheries activities, improving the condition of fishing grounds, stock enhancement of fisheries resources, and promotion of resources management though a participatory approach.

International assistance for the development of the marine fisheries sector in Cambodia has been apparently limited, except in some aspects like conservation of coastal fisheries resources. On the contrary, the inland fisheries sector has received assistance from various countries. With respect to the marine aquaculture sector in which less technical development has been observed, aquaculture operators primarily depend on fingerlings captured from the wild and imported from neighboring countries. This is due to the fact that domestic hatcheries have not yet been established in Cambodia except for a recently opened Marine Aquaculture Research and Development Center that is not yet fully operational. The collection of wild seeds for aquaculture farming has resulted in the acceleration of pressure on the natural fisheries resources. In addition, inadvertent outbreaks of diseases in cultured fish and shrimps whose seeds or fingerlings originated from the wild likewise hinder the development of the marine aquaculture sector despite the promotion of marine aquaculture being identified as a priority area in the fisheries sector of Cambodia (Viseth and Pengbun, 2005).

Current status of shrimp farming

Shrimp farming in Cambodia began in 1989 and has significantly expanded since 1991. The shrimp farming activity has been carried out along the coastline of Cambodia, i.e. in the four coastal provinces namely Kampot, Kep, Preah Sihanouk Ville, and Koh Kong. Two main species of penaeid shrimps have been popularly cultured, i.e. black tiger shrimp (*Penaeus monodon*) and whiteleg shrimp (*P. vannamei*). Moreover, two types of culture system, i.e. extensive and intensive, have been practiced in these farms over the past several years (Hav and Leap, 2005).

Extensive shrimp farming system has been mostly practiced by farmers in Kampot and Preah Sihanouk Ville provinces. In general, shrimp ponds were constructed close to the mangrove areas with some ponds containing mangroves within the pond. The average size of these ponds ranges from 5-10 ha. These extensive shrimp farms depend mainly on natural food propagated in the pond and tidal water replenishment. It requires a low cost investment for pond construction, preparation and farm operation. In addition, the use of commercial feeds, water aeration, and control of predators for one full cycle of shrimp cultivation or until harvesting are not required. Shrimp's stocking density usually ranged from 5,000 to 20,000 larvae/ha; however, productivity remains low at less than 100 kg/ha per year.

On the contrary, intensive cultivation of shrimps has been mainly practiced in Koh Kong province. Intensive shrimp farming system was first introduced by a Thai shrimp farmer and businessman. It requires high cost of investment for farm establishment, pond construction and farm operation. Shrimps need to be fed with pellet or formulated diet and water aeration and regular water exchange are required. The main species being cultured intensively in this area is *P. monodon* at stocking density ranging from 300,000 to 500,000 postlarvae/ha. Notably, productions of the newly established farms have reached 7-8 MT/ha per crop.

Current status of shrimp diseases

The occurrence of white spot disease (WSD), monodon baculovirus disease, and yellow head disease caused respectively by white spot syndrome virus (WSSV), monodon baculovirus (MBV), and yellow head virus (YHV) was first reported in 1999 among cultured *P. monodon* in Koh Kong province causing a number of farmers to stop the intensive cultivation of black tiger shrimp. Levels II and III diagnostic procedures have been used to identify the above shrimp viruses, although polymerase chain reaction (PCR) method is the only Level III diagnostic procedure currently available to detect WSSV in shrimp (Table 1) (Racy, 2004). The Fish Health Laboratory of the Fisheries Administration (FHL-FA) has started to develop capability in virology, especially on virus isolation using the cell culture technique. A surveillance was conducted by FAIEX II/JICA project from 18 to 22 June 2015 in the four coastal provinces of Cambodia namely Kep, Kampot, Preah Sihanouk Ville, and Koh Kong. The result of the survey revealed that most of the farmers had actually experienced outbreaks or shrimp mortalities particularly encountered during the first 35 days of culture from 2011 until 2013. Moreover, farmers noted that affected shrimps (*P. monodon*) have empty gut, loose and pale shells, and swam on the water surface. The outbreak was speculated to be due to acute hepatopancreatic necrosis disease (AHPND), however, diagnostic tests have not been carried out to ascertain the etiology of the disease. Aside from this, farmers also experienced serious outbreaks of WSD. Because of these unwarranted disease outbreaks that led to serious economic losses, since 2014, most of the shrimp farmers shifted to grouper and sea bass culture. To date, only a small proportion of shrimp farmers have ventured into extensive shrimp farming with approximately 10 ha of shrimp areas currently in operation.

Table 1. List of government, private, and university-based Fish Health Laboratories and their level of diagnostic capability.

Name of Laboratory	Level of Diagnosis	Address Location
Department of Fisheries	*Level II* (1) Bacteriology (2) Mycology (3) Parasitology (4) Histopathology	#186 Preah Norodom Blvd, Sangkat Tonle Bassac, Khan Chamcarmon, Phnom Penh Cambodia, P.O Box582 Tel.:855 23 215 470
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Pasteur Institute	*Levels II and III* (1) Bacteriology (2) Mycology (3) Parasitology (4) Histopathology (5) Virology (6) Immunology	Monivong Blvd, Khan Toul Kork, Phnom Penh, Cambodia Tel.:855 12 814 276

Economic impact

A review of shrimp farming showed that the intensive shrimp farming in Koh Kong province increased up to 1,000 ha in 1995 but eventually started to decline with the onset of WSD outbreaks. This viral disease of shrimp has by far been identified as the most serious threat faced by the shrimp farmers in Cambodia causing economic losses of approximately USD 14.5 million per year (Touch Seang Tana, 2002). From 2002 until 2015, pertinent data on shrimp farming in Cambodia were scarce.

Way forward

In terms of technical development, the marine aquaculture sector in Cambodia has not relatively reached the level of inland aquaculture. Shrimp farmers still depend on postlarvae captured from the wild and imported from neighboring countries resulting in the acceleration of pressure to the natural fishery resources and inadvertent introduction of transboundary pathogens leading to inevitable occurrences of disease epizootics (Lang, 2015). Diseases have persistently remained as a major constraint besetting the shrimp industry of Cambodia. To mitigate the negative impact of shrimp diseases and promote the expansion of the country's shrimp industry, strategies aimed at sustainability should be implemented including the (a) development of a national reporting systems for aquatic animal diseases, especially shrimp diseases; (b) capacity building on shrimp diseases, procedures for monitoring and disease surveillance; (c) creation of national guidelines on *Good Aquaculture Practice* (GAP) in shrimp farming; (d) establishment of sub-research center for marine aquaculture development and extension services in all coastal provinces that have potential for marine aquaculture development, particularly shrimp farming and concomitant funding support for research; (e) establishment of local hatcheries in all coastal provinces, i.e. *Seed Production Decentralization* which is a key point in reducing time and distance of transportation, and ease in the provision of seeds to local farmers and provision of hands-on trainings for farmers; and (f) strengthening collaborations among provincial officers, researchers and farmers' network.

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Current Status of Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Diseases of Farmed Shrimps in Indonesia

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Abstract

Transboundary diseases have been a constant challenge for the aquaculture industry in Indonesia. In spite of this, Indonesian aquaculture has experienced a steady growth since 2010. Early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) is a serious emerging transboundary disease of cultured shrimp that has not been reported in Indonesia. On the contrary, hepatopancreatic microsporidiosis (HPM) was first detected in 2015. Other previously reported transboundary diseases of shrimp and fish include white spot disease (WSD), Taura syndrome and infectious myonecrosis (IMN), and viral nervous necrosis (VNN) and koi herpesvirus (KHV), respectively. These diseases have been included in the surveillance program conducted in 2016. To avert the spread of these transboundary pathogens in the Indonesian aquaculture facilities and natural waters, competent authorities have been tasked to implement stringent control measures including government policy and regulation, active and passive surveillance, and strengthening farmers' and stake holders' awareness of the importance of disease control and health maintenance.

Introduction

Aquaculture has contributed significantly to the Indonesian economy for over 4 decades. Currently, Indonesia is one of top farmed food fish-producing countries in the world (FAO 2014). The Ministry of Marine Affairs and Fisheries (MMAF) of Indonesia has provided supports, among them, through regulations of disease control and sustainable aquaculture, technical assistance for various stakeholders, working closely with experts from universities to achieve the national aquaculture production target. Moreover, rigorous efforts aimed at increasing farmers' awareness on transboundary diseases and best manufacturing practices (BMPs) have been aggressively instituted over the past years. As a result, aquaculture production increased significantly from 6.28 million metric tons (MT) in 2010 to 14.36 million MT in 2014 (Table 1). For 2016, the annual production target has been projected at 19.45 million MT, with penaeid shrimps anticipated to contribute approximately 0.93 million MT. Although seaweed represents the major aquaculture commodity in terms of production, shrimps have been an essential part of the aquaculture industry in Indonesia chiefly because of the price that they command in the international market (Table 2).

Diseases have been the major challenge faced by the aquaculture sector in Indonesia. Over the past 10 years, the Government of Indonesia, through MMAF and various stakeholders, has been exerting tremendous efforts to reduce the impact of transboundary diseases, with adequate results. The most important diseases of cultured penaeids so far documented include white spot disease (WSD) and infectious myonecrosis (IMN) caused by white spot syndrome virus (WSSV) and infectious myonecrosis virus (IMNV), respectively. To date, acute hepatopancreatic necrosis disease (AHPND), a newly reported emerging disease of cultivated shrimp, has not been encountered in Indonesia. However, hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) was detected in some areas in 2015. Major fish diseases include koi herpesvirus disease caused by koi herpesvirus (KHV) and viral nervous necrosis (VNN) caused by betanodaviruses. This paper reports the current status of newly emerging and previously reported transboundary diseases of aquacultured organisms in Indonesia with particular emphasis on emerging diseases of penaeid shrimps.

Status of newly emerging transboundary diseases of penaeid shrimps

Acute hepatopancreatic necrosis disease (AHPND)

AHPND first occurred in China in 2009 and has since been reported in Viet Nam (2011), Malaysia (2011), Thailand (2012), México (2013), and the Philippines (2015) (Tran *et al.*, 2013; Joshi *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; dela Peña *et al.*, 2015). Indonesia is one of the major shrimp-producing countries in Asia in which AHPND outbreak has not yet been encountered. Competent authorities have

Table 1. Aquaculture Production of Indonesia from 2010 to 2014.

No.	Commodities	Production (MT)				
		Year				
		2010	2011	2012	2013	2014
1	Shrimp					
	Tiger shrimp	125,519	126,157	117,888	171,583	131,809
	White shrimp	206,578	246,420	251,763	390,278	442,380
	Freshwater prawn	1,327	1,386	1,721	3,387	1,809
	Others	47,548	27,191	44,331	73,707	63,371
	SUB TOTAL	**380,972**	**401,154**	**415,703**	**638,955**	**639,369**
2	Seaweed					
	Gracilaria	515,581	630,788	776,166	975,211	1,105,529
	Cottonii	3,399,436	4,539,413	5,738,688	8,335,663	8,971,463
	SUB TOTAL	**3,915,017**	**5,170,201**	**6,514,854**	**9,310,874**	**10,076,992**
3	Fish					
	Milkfish	421,757	467,449	518,939	627,333	631,125
	Grouper	10,398	10,580	11,950	13,464	13,346
	Asian Sea bass	5,738	5,236	6,198	6,735	5,447
	Molluscs	58,079	48,449	19,472	29,091	44,394
	Crabs	9,557	8,153	14,330	11,911	13,606
	Common Carp	282,695	332,206	374,366	412,703	434,653
	Tilapia	464,191	567,078	695,063	914,778	999,695
	Catfish	242,811	337,577	441,217	543,774	679,379
	Pangasius	147,888	229,267	347,000	410,883	418,002
	Giant Gourami	56,889	64,252	84,681	94,605	118,776
	Others	281,931	287,360	231,778	285,800	284,348
	SUB TOTAL	**1,981,934**	**2,357,607**	**2,744,996**	**3,351,077**	**3,642,769**
	TOTAL	**6,277,923**	**7,928,962**	**9,675,553**	**13,300,906**	**14,359,129**

declared Indonesia as an AHPND-free country in the region since 2013 based on the decree of the Director General of Fish Quarantine and Inspection Agency No. 130/KEP-BKIPM/2013.

AHPND field observation

The etiological agent of AHPND is a virulent strain of *Vibrio parahaemolyticus* that contains pVPA3-1 plasmid producing PirA and PirB toxins. Because the plasmid contained in *V. parahaemolyticus*-causing AHPND (VP_{AHPND}) is transmissible coupled by the fact that it is commonly found in brackishwater environments, MMAF has been conducting field observations integrated with the surveillance program for WSSV and IMNV in North Sumatra and Lampung provinces since 2013 and 2015, respectively. *P. vannamei* broodstocks, larvae and juveniles, as well as polychaetes (*Nereis* sp.), clams and *Artemia* which are fed to the shrimps are covered by the surveillance for these aforementioned shrimp pathogens.

Pond managers were interviewed for disease occurrence with emphasis on AHPND, i.e. nonspecific mortality during the first 50 days of culture. Specimens for diagnostics consisted of juvenile *P. vannamei* from intensive and traditional ponds. Isolation and detection of AHPND causal agents were conducted according to methods outlined in the OIE manual. Bacterial identification was done using API 20NE (bioMerieux). *V. parahaemolyticus* isolates were tested for the presence of specific plasmid of VP_{AHPND} using the primer set AP2, AP3, AP4, Tox R and VpPir A and VpPir B. Pathological changes in the hepatopancreas

Table 2. Indonesian aquaculture production value.

No.	Commodities	Value in Indonesian Rupiah (Rp) 1,000,000,000 Year 2010	2011	2012	2013	2014
1	Shrimp					
	Tiger shrimp	7.20	7.24	6.86	6.97	9.09
	White shrimp	8.19	10.24	10.38	17.84	25.87
	Freshwater prawn	0.08	0.04	0.19	0.18	0.09
	Others	1.40	0.60	1.03	1.82	2.04
	SUB TOTAL	**16.86**	**18.11**	**18.46**	**26.80**	**37.09**
2	Seaweed					
	Gracilaria	1.52	0.66	1.31	1.40	1.22
	Cottonii	10.23	10.23	10.28	20.31	23.38
	SUB TOTAL	**11.75**	**10.90**	**11.59**	**21.71**	**24.60**
3	Fish					
	Milkfish	4.89	6.75	8.42	8.97	9.58
	Grouper	2.27	1.16	1.56	1.35	1.46
	Asian Sea bass	0.18	0.19	0.26	0.28	0.21
	Molluscs	0.26	0.18	0.13	0.04	0.20
	Crabs	0.27	0.23	0.45	0.43	0.50
	Common Carp	5.84	6.51	7.26	6.60	9.58
	Tilapia	9.52	9.47	10.70	12.39	18.03
	Catfish	2.75	3.93	5.26	7.26	10.32
	Pangasius	2.48	3.30	4.62	4.38	6.45
	Giant Gourami	1.65	1.51	2.54	2.13	3.32
	Others	4.70	4.31	4.67	3.70	6.36
	SUB TOTAL	**34.81**	**37.54**	**45.87**	**47.52**	**66.01**
	TOTAL	**63.42**	**66.55**	**75.92**	**96.04**	**127.70**

1 USD = 13,070 Rp

and intestines of shrimps were examined by histopathology. In addition, selected *V. parahaemolyticus* strains were subjected to 16S rRNA sequencing. The sequence data obtained from these strains were compared with the sequence data in the National Center for Biotechnology Information (NCBI) database. In addition, seventeen specimens composed of *P. vannamei* juveniles obtained from shrimp ponds in North Sumatra with white feces disease (WFD) were likewise tested in 2013. Isolation and subsequent identification were done as described above. Other *Vibrio* species including *V. vulnificus* and *V. furnissii* were also isolated in some shrimps examined. Although histopathological examinations of some shrimp samples exhibited sloughing of the hepatopancreatic tubules, they were however positive for Cowdry type A inclusion bodies indicative of WSSV infection. Fortunately, all shrimps collected during the active surveillance conducted from 2013 to 2015 were by far negative for AHPND by AP2, AP3 and AP4 PCR methods (Table 3).

White feces disease (WFD)

White feces disease outbreaks occurred in some shrimp cultivation areas in Indonesia including East Java, DI Yogyakarta, North Sumatra and Lampung in 2014. *Vibrio parahaemolyticus* obtained from 19 samples with WFD across Indonesia were examined by 16S rRNA sequencing. As a result, *V. parahaemolyticus* strains that are closely related to VP_{AHPND} were found to be present in Indonesia but fortunately they turned out to be AHPND-negative by PCR for both the plasmid and toxin, clearly indicating that AHPND is still absent in Indonesia,

Hepatopancreatic microsporidiosis (HPM)

Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) was detected in Indonesia in 2015. EHP-positive shrimp was found in East Java (Banyuwangi and Situbondo), Lampung (Rawa Jitu), and Bali (Nagara,). However, at this stage the prevalence of HPM in Indonesia has yet to be determined because a systematic survey for EHP has not been conducted yet. So far, no overt clinical signs have been noted in any of the shrimp samples examined. In September 2015, farmers in Situbondo, East Java, reported concerns regarding the slow growth of their shrimps. At that time, shrimps cultivated in some ponds exhibited white feces disease. However the association between these two factors, i.e. slow growth and white feces disease, still needs to be thoroughly investigated.

EHP was detected in the hepatopancreas and intestines of *P. vannamei* samples by PCR. However, the source and route of infection remains unclear. Control measures that are currently being undertaken involve rigorous

Table 3. Results of surveillance and monitoring for transboundary diseases of penaeid shrimps in Indonesia from 2013 to 2015.

No.	Location	Disease			
		WSSV	IMNV	AHPND	HPM
1	North Sumatra	+	+	-	ND*
2	Lampung	+	+	-	+
3	Banten	+	+	-	ND
4	West Java	+	+	-	ND
5	Central Java	+	-	-	ND
6	East Java	+	+	-	+
7	West Kalimantan	+	+	-	ND
8	East/North Kalimantan	+	+	-	ND
9	South Sulawesi	+	-	-	ND
10	Bali	+	+	-	+
11	West Nusa Tenggara	+	-	-	ND

**Not done*

biosecurity and BMPs, lowering stocking density from 150 to 80 larvae/m^2, proper pond preparation, screening of broodstocks and larvae for EHP by PCR method, and using probiotics. HPM has been included in the surveillance program in 2016.

Action plans

Indonesia's competent authorities shall strictly implement pertinent regulations to maintain its AHPND-free status. Action plans in place to prevent the introduction of AHPND and other transboundary diseases of penaeid shrimps and fish into the Indonesian waters are enumerated below. These action plans have been rigorously disseminated to 12 major shrimp production areas, i.e. North Sumatera, Lampung, Banten, West Java, Central Java, DI. Yogyakarta, East Java, West Kalimantan, East Kalimantan, South Sulawesi, Bali, and Nusa Tenggara Barat through public-private partnership consultations, meetings and fora.

(1) Government regulations issued by the Ministry of Marine Affairs and Fisheries (MMAF) on the prevention of AHPND introduction to Indonesia:

   (a) MMAF Decree declaring the prohibition of shrimp and polychaete importation from or transit in countries with confirmed case of AHPND outbreak;

   (b) MMAF Decree on the institution of EMS/ AHPND Task Force;

   (c) Director General (DG) of Aquaculture's Circulation Letter on early mortality syndrome (EMS) prevention; and

   (d) DG of Aquaculture Decree pronouncing the prohibition of probiotic importation from or transit in countries with confirmed case of AHPND outbreak.

(2) Ongoing active and passive surveillance on AHPND, HPM, WSD, IMN in major shrimp production areas and hatcheries in Indonesia. Passive surveillance has been successfully implemented by working closely with the stakeholders, shrimp culture companies, and shrimp farmer associations such as the Shrimp Club Indonesia. Specimens for pathogen detection include shrimps of various stages, broodstocks, and natural food for broodstocks and larvae (e.g. polychaetes, *Artemia*, clams, etc.).

(3) Strengthening of farmers and shrimp hatchery operators' knowledge on transboundary disease prevention and control through strict adherence to biosecurity protocols and BMPs, and judicial use of chemicals and antibiotics in aquaculture.

(4) Eradication of AHPND-infected shrimp population if deemed required and necessary by competent authorities to prevent the spread of AHPND.

Updates on previously reported transboundary diseases of penaeid shrimps

White spot disease (WSD) and infectious myonecrosis (IMN)

Over the last 5 years, two major transboundary disease outbreaks of penaeid shrimps have been reported in Indonesia: white spot disease and infectious myonecrosis caused by WSSV and IMNV, respectively. Although shrimp production has started to recover from the devastating impact of WSD since 2002, WSD has remained as one of the most devastating diseases of farmed shrimp in Indonesia. The surveillance program will be continued until 2017. This is in line with our ultimate goal of declaring certain areas in Indonesia as WSSV- and IMNV-free.

Active surveillance for WSSV and IMNV was conducted following the method of Cameron (2002) and manual for aquaculture disease surveillance of MMAF. Active surveillance has been so far conducted in 11 provinces constituting the major shrimp farming areas in Indonesia. In average, 150-200 shrimp samples were obtained from 150 ponds (10% of the farms per area) per sampling. The surveillance was conducted twice a year when the cultured shrimps were at their most susceptible stage (30-40 days of culture). In addition, pond management and farmers knowledge and awareness about the impacts of WSD, IMN, AHPND and other transboundary diseases of penaeid shrimps in general was likewise assessed using a questionnaire.

The results of the surveillance showed that WSSV and IMNV were present in the sampling areas examined (Table 3). The prevalence of WSSV-positive shrimp varied among the sampling areas and time of specimen collection. Interestingly, the presence of WSSV in collected samples did not always correlate with WSD outbreak. In general, the prevalence of IMNV in the sampling areas examined was lower than that of WSSV.

Impact of infectious diseases on aquaculture species

Disease outbreaks in hatcheries and grow-out ponds have remarkably reduced aquaculture production and productivity due to mortality, slow growth, low feed conversion and efficiency. In addition, contamination of aquaculture environments with infectious pathogens, inefficiency in the use of fishery resources, decrease in product quality which could result in loss of potential market, decrease in price and revenue due to the slowing down of aquaculture business intensification and expansion, and unsustainability have likewise resulted in reduced aquaculture production and productivity. Economic losses due to fish diseases consequently gave rise to unpaid bank debts, unemployment, poverty, and decline on investments by feed industries and processing plants among others. Based on the result of the epidemiological analysis undertaken by MMAF and universities in 2011, economic losses due to infectious and non-infectious diseases of major aquaculture species in Indonesia could be billion rupiah (13,070 Indonesian rupiah = 1 USD) per annum. It is also worth noting that despite stringent efforts employed to prevent and control diseases of major aquaculture species through several approaches enumerated in the action plans above, losses ascribed to infectious diseases still appeared to be economically significant.

The estimated annual financial losses were based on the epidemiological data of persistently devastating diseases of major aquaculture commodities in Indonesia (Table

Table 4. Estimated annual financial losses due to aquatic diseases.

Culture environment	Disease	Main commodity affected	Mortality range (%)	Loss estimation/ year (million rupiah)
Freshwater	Koi herpesvirus (KHV)	Gold fish and koi	30-80	50,000
	Motile aeromonad Septicaemia (MAS)	All types of freshwater fish	20-100	400,000
	Streptococcosis	Tilapia	20-50	15,000
	Mycobacteriosis	Gouramy	10-50	7,500
	Ichthyophthiriosis	All types of freshwater fish	25-100	50,000
	Others	All types of freshwater fish	10-100	150,000
SUB TOTAL				**672,500**
Brackishwater	White spot diseases (WSD)	Tiger and white shrimps	30-100	2,500,000
	Infectious myonecrosis (IMN)	Tiger and white shrimps	10-60	1,250,000
	Vibriosis	Tiger and white shrimps	10-40	100,000
	Others	Tiger and white shrimps	10-25	50,000
SUB TOTAL				**3,900,000**
Marine	Viral nervous necrosis (VNN)	Grouper	10-100	250,000
		Seabass	30-70	150,000
	Vibriosis	Grouper and seabass	10-80	150,000
	Ice-ice	Seaweed	10-30	50,000
	Others	Cultured marine fish	10-50	100,000
SUB TOTAL				**700,000**
TOTAL				**5,272,500**

1 USD = 13,070 Rp

4). The economic losses have been estimated at 5.2 trillion Indonesian rupiah.

Way forward

The surveillance program on WSSV, IMNV, and other transboundary pathogens which was instituted in 2013 will be continued until 2017. Notably, MMAF has been actively supporting the development of vaccines against viral diseases of fish (KHV disease and VNN) and shrimps (WSSV infection) and other equally important diseases implicated in serious mortalities of freshwater fish species such as aeromonad septicemia, streptococcal infection, and mycobacteriosis, among others. Field trials of the VNN and bacterial vaccines in marine and freshwater fish species, respectively, will be conducted in 2016. Laboratory data pertinent to the management of IMN and WSD in penaeid shrimps, i.e. through water quality manipulation, have already been generated. However, its practical application in the field has yet to be evaluated. In addition, investigation of the antimicrobial potential of local herbs in treating bacterial diseases of shrimp and fish are being likewise undertaken.

To keep abreast with the impacts of emerging and persistent disease problems on economically important aquaculture species, our future researches will focus on (1) the effect of chemicals and antibiotics not only on cultivated aquatic species but as well as in non-target resident organisms in the pond such as polychaetes, snails, and crab; (2) long-term effects and sustainability of using indigenous and imported probiotics particularly in ponds equipped with plastic liners; (3) establishment of efficient and economically sound approaches geared at increasing productions of SPF shrimp seeds; (4) domestication and diversification of new species; (5) development of effective and economically viable vaccines for viral and bacterial diseases of fish and crustaceans; and (6) elucidation of the mechanisms behind the behaviour of infectious disease agents in asymptomatic (carriers) hosts and vectors. The outputs of these researches will be pivotal in the formulation of pragmatic and economically viable strategies that will propel the institution of a more holistic and sustainable aquaculture sector.

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Important Diseases and Practical Control Measures in Shrimp Culture in Japan

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Abstract

The gross product from sea culture in Japan was about USD 3.4 billion in 2013 with kuruma shrimp *Marsupenaeus japonicus* constituting 2% of the total production. In recent years, annual shrimp production has reached about 1,600 metric tons (MT) and 99% of the species produced comprised of *M. japonicus*. Kuruma shrimp is highly traded at market price of USD 40-60 per kg. At present, 65% of cultured kuruma shrimp are produced in Okinawa and Kagoshima prefectures. To increase or maintain kuruma shrimp fishery in the natural environment, 200 million fry have been annually released into the sea along the coast of Japan. In addition, whiteleg shrimp *Penaeus vannamei* has also been produced in a private farm since 2007 with an annual production of approximately 40 MT. Recently, the number of farms that ventured into whiteleg shrimp culture has increased.

The most serious obstacle faced by the kuruma shrimp industry in Japan in the 1990s was the outbreaks of white spot disease (WSD) caused by white spot syndrome virus (WSSV). The seed production of kuruma shrimp has been dependent on captured wild broodstocks. However, broodstocks obtained from the wild could be carriers of WSSV that may vertically transmit the virus to fertilized eggs. To prevent the spread of WSD, measures for disinfecting WSSV-infected fertilized eggs of shrimp and detection of the causative virus by polymerase chain reaction (PCR) have been developed. Lately, with the application of an improved technology for broodstock rearing, production losses ascribed to WSD have significantly decreased because majority of the hatcheries have been using specific WSSV-free shrimps.

In the last decade, in kuruma shrimp hatcheries and grow-out ponds, vibriosis due to *Vibrio penaeicida* has been frequently encountered. Fusariosis caused by *Fusarium solani* is also common. The first cases of vibriosis and fusariosis in Japan occurred in 1973 and 1972, respectively. Taking the case of Okinawa prefecture as example, production losses in 2005 due to vibriosis and fusariosis were 66% and 34%, respectively. Cleaning of the pond bottom prior to the commencement of grow-out culture has been recommended to prevent vibriosis. Some antibiotics have also been orally administered to reduce mortality of shrimps affected by vibriosis. However, farmers have been properly cautioned regarding their use as emergence of antibiotic-resistant bacteria could consequently arise. Because no practical treatment for fusariosis is currently available, farmers either dispose or harvest infected shrimps followed by disinfection of ponds with 10 ppm chlorine.

Acute hepatopancreatic necrosis disease (AHPND) has not yet been detected in Japan. As kuruma shrimp is also susceptible to the disease, the Government of Japan plans to designate AHPND as *Specific Disease*. Under Japanese law, enlistment of AHPND as a quarantinable disease will also be instituted to prevent the introduction of this disease into Japanese hatcheries and grow-out facilities. However, in case an AHPND outbreak inadvertently occurs, Prefectural Fisheries Experimental Stations (PES) could immediately conduct preliminary diagnosis using the AP4 polymerase chain reaction (PCR) method. In addition, confirmatory diagnosis using AP4 nested PCR method and sequencing could be conducted at the National Research Institute of Aquaculture (NRIA), a central laboratory for aquatic animal health. NRIA's task is not only to perform confirmatory diagnosis but likewise disseminate diagnostic techniques to the staff of the National Quarantine and PES.

Introduction

The origin of shrimp farming could be dated back to the 1930s when the kuruma shrimp spawned in captivity and was cultured for the first time in Japan (Hudinaga, 1942). A small shrimp farming industry emerged in Japan in the 1960s. Later that year, commercial shrimp farming proliferated (Hudinaga and Kittaka, 1975). In recent years, annual production of shrimp has reached approximately 1,600 metric tons (MT) with 99% of the species produced comprised of kuruma shrimp (FAO, 2012). However, the occurrence of infectious diseases attributed to vibriosis, fusariosis and white spot disease (WSD) has, over the past years, deterred the sustainability of the shrimp industry in Japan (Satoh, 2012).

The introduction of WSD caused by white spot syndrome virus (WSSV) through shrimp larvae from overseas in 1993 had caused serious economic losses in Japan's shrimp industry (Inouye *et al.*, 1994; Nakano *et al.*, 1994). Because the primary infection route of the causative virus was identified to be via vertical transmission, i.e. from spawners to larvae (Lo and Kou, 1998), eggs obtained from broodstocks after spawning were screened by polymerase chain reaction (PCR). In addition, fertilized eggs were washed with disinfectant prior to hatchery rearing (Mushiake *et al.*, 1999). In spite of these interventions, in some cases, occurrence of WSD was encountered during culture due to latent virus infection because viral loads in shrimps with inapparent infection could be extremely low and very difficult to detect even by sensitive methods such as real-time PCR.

WSSV-free-cultured shrimps have been recently available as broodstocks for captive breeding in several shrimp hatcheries chiefly due to improvements in the rearing techniques (Sugiyama, 2007). As a result, production losses resulting from WSD have markedly decreased over the past several years. Okinawa prefecture is the largest shrimp producer in Japan wherein approximately 70% of larvae are obtained from mature captive broodstock (Matsumoto, 2015). However, some hatcheries are still dependent on wild caught broodstock. Aside from WSD, vibriosis and fusariosis could infect penaeid shrimps cultivated in grow-out ponds.

Acute hepatopancreatic necrosis disease (AHPND) caused by unique strains of *V. parahaemolyticus* has not yet been detected in Japan. However, the domestic shrimp price in Japan soared to USD 15.5 from USD 10.5, when the import volume from Thailand and Malaysia decreased due to AHPND outbreaks. Notably, importation of shrimps from Viet Nam and India momentarily increased (Editorial office, 2015). As kuruma shrimp has been demonstrated to be susceptible to the disease via experimental infection, the Government of Japan plans to designate AHPND as *Specific Disease*. Under Japanese law, enlistment of AHPND as a quarantinable disease will also be instituted to prevent the introduction of this disease into Japanese hatcheries and grow-out facilities. However, in the event that an AHPND outbreak inadvertently occurs in Japan, Prefectural Experimental Stations (PES) could immediately conduct the preliminary diagnosis using the AP4 polymerase chain reaction (PCR) method (Dangtip *et al.*, 2015). In addition, confirmatory diagnosis using AP4 nested PCR method coupled with sequencing could be conducted at the National Research Institute of Aquaculture (NRIA), a central laboratory for aquatic animal health.

Major diseases of kuruma shrimp and their diagnostic methods

Three major infectious diseases, i.e. vibriosis, fusariosis and WSD of penaeid shrimps have been mainly documented among cultivated kuruma shrimps in Japan. While considerable economic losses caused by infectious diseases were encountered in the 1990's, these diseases occur sporadically at present. From a total production valued at about USD 79.8 million in 2008, production losses were estimated at USD 8.8 million with vibriosis constituting 53% of the losses, followed by WSD (31%), and fusariosis (16%). However, economic losses due to fusariosis has gradually increased in the last decade.

Almost all life stages of kuruma shrimp are susceptible to WSD, i.e. from juveniles to mature adults. WSD has not only caused serious economic losses to the shrimp aquaculture industry, but also huge troubles in stock enhancement of kuruma shrimp for decades. Kuruma shrimps infected with WSD exhibit embedded white spots within the exoskeleton, rapid reduction of food consumption, and lethargic behavior. Nested PCR or real-time PCR methods have been employed to diagnose the disease.

Vibriosis has been observed in shrimps weighing 1- 45 g with the most sensitive stage noted in shrimps with body weights ranging from 15-25 g. *Vibrio penaeicida* infection in cultured kuruma shrimp was first observed in 1973 (Ueda, 1973) and has been the primary cause of shrimp losses until the emergence of WSD in Japan in 1993. Affected shrimps exhibit small black spots in the gill filaments. In addition, lymphoid organs become enlarged with brown spots. Diagnostic methods include bacterial isolation on marine agar or thiosulfate-citrate-bile salts-citrate (TCBS) agar followed by the identification of isolated bacteria using anti-serum slide aggregation test or PCR.

Only kuruma shrimp is so far sensitive to *Fusarium* spp. Fusariosis has been documented in cultivated shrimps both in hatcheries and grow-out ponds. In addition, mortality in younger stages, i.e. from nauplius to postlarvae 10 has been noted to be higher compared to those cultivated in grow-out ponds during the latter stages of culture. Fusariosis first occurred in 1972 (Egusa and Ueda, 1972) and has been persistently observed up to the present. The gills of shrimps infected with fusariosis turn blackish in color when viewed under the microscope due to the presence of fungal hyphae. *Fusarium* spp. can be isolated from the infected gills using Mycosel agar or artificial seawater yeast extract peptone glucose (PYGS) agar. Additionally, *Fusarium* spp. can be identified microscopically based on the morphological characteristics of the hyphae and conidia.

Disease prevention and control

The primary infection route of WSD in hatcheries has been ascribed to the vertical transmission of WSSV from spawners to eggs and larvae. This is supported by the fact that wild shrimps used for induced spawning have

Table 1. Prevalence of white spot syndrome virus (WSSV) in wild mature kuruma shrimp from 1996 to 1998.

Sex	Organs	Prevalence	Number of WSSV positive/total
Female	Gonad	10.1%	96/955
	Stomach	7.3%	70/955
	Hemolymph	5.8%	52/892
Male	Gonad	4.8%	15/314
	Stomach	6.7%	21/314
	Hemolymph	0.0%	0/314

Table 2. Frequency of white spot disease (WSD) occurrences in the seed production of kuruma shrimp at National Research Institute of Aquaculture from 1996 to 1999.

Year	Selection of spawner by PCR		Disinfection treatment of eggs	Number of cases with WSD/Total (%)
	Target Organ	Timing of sampling		
1996	Ovary	not done	not done	8/14 (57.1%)
1997	Ovary	before spawning	with iodine	2/23 (8.7%)
1998	Receptaculum seminis	after spawning	with iodine	0/11 (0%)
1999	Receptaculum seminis	after spawning	with iodine	0/16 (0%)

been often infected by WSSV (Table 1). Thus, to inhibit the vertical transmission of WSSV, eggs are selected based on WSSV detection in broodstocks by PCR after spawning. The target organ for PCR is the receptaculum seminis. In addition, the fertilized eggs are disinfected with povidone iodine (5 mg/l for 5 min). Frequency of WSD occurrences in the seed production of kuruma shrimp at NRIA from 1996 to 1999 is shown in Table 2. Our established protocol involving a combination of WSD negative spawner selection by PCR that detects the virus from the receptaculum seminis and disinfection of fertilized eggs with iodine could successfully prevent larvae and post-larvae from being contaminated with WSSV. In addition, to prevent the horizontal transmission of WSSV, seawater is treated by UV irradiation before being pumped into the rearing tanks of shrimp larvae or juveniles. Stable production of specific-pathogen-free shrimp has been accomplished by these countermeasures against WSSV transmission. Control measures aimed at preventing WSD in hatcheries are shown in Figure 1. Additionally, horizontal transmission of WSSV among pond cultivated shrimps has been primarily identified to be via cannibalism and the waterborne route. Vaccines (subunit and DNA) targeting major envelope proteins such as VP28 of WSSV in penaeid shrimp have been shown to elicit good protection against WSD (Satoh *et al.*, 2008). Unfortunately, field trial has not yet been conducted due to patent limitation and high costs.

Vibriosis often occurs when the sand in the pond bottom contains excessive organic matter. Thus, to prevent this condition, the pond bottom should be turned over after harvesting shrimp or the sand should be renewed, if possible. While oxytetracycline hydrochloride or oxolinic acid have been used to treat vibriosis in kuruma shrimp, no approved antibiotics for shrimp vibriosis is currently available in Japan. Immunostimulants such as beta-1,3-glucan and peptidoglycan have been proven effective in reducing shrimp mortality due to vibriosis (Itami *et al.*, 1989; Takahashi *et al.*, 1995), but its application is seemingly not economically practical. No practical treatment for fusariosis is currently available; hence, farmers either dispose or harvest infected shrimps followed by disinfection of pond water with 10 ppm chlorine.

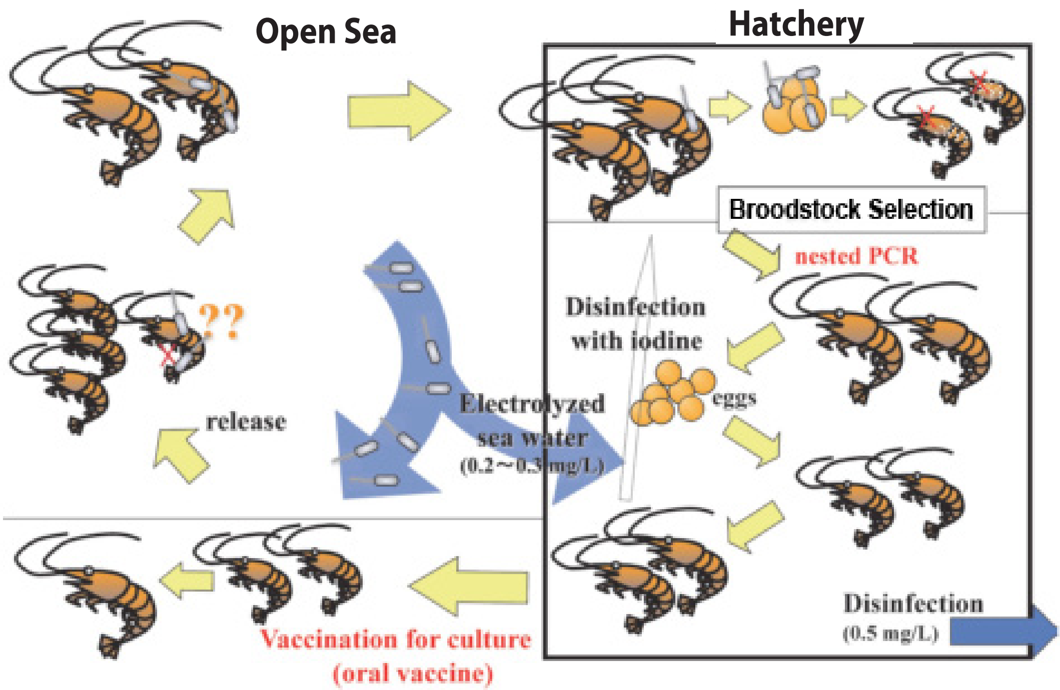

Figure 1. Control measures to prevent shrimp from viral contamination (extracted from Bull. Fish. Res. Agen. No. 36, 57-106, 2012).

Preventive measures against the spreading of important diseases of aquacultured organisms in Japan have been implemented according to two Japanese laws, i.e. *Sustainable Aquaculture Production Assurance Act* (Law No.51 of 1999, amended in Law No.69 of 2014) and *Act on the Protection of Fisheries Resources* (Law No.313 of 1951, amended in Low No. 70 of 2015). The former law clearly stipulates that necessary measures should be aimed at preventing the spread of specified infectious diseases among farms in order to maintain the stable supply of aquatic products. On the other hand, the latter stipulates that quarantine procedures for imported aquatic animals should be strictly followed. The laws designated 11 diseases as *Specified Diseases*, including 5 shrimp diseases. At the moment, the Ministry of Agriculture, Forestry and Fisheries (MAFF) is planning to increase the number of the *Specified Diseases* to a total of 24 diseases including AHPND and 9 other shrimp diseases (Table 3).

The Animal Quarantine Service (AQS) of the MAFF performs import quarantine inspection at 30 ports located around Japan. The staff of AQS inspect documents for imports including heath certificate and confirm the number/condition of imported animals. When necessary, the animals are kept at a designated facility during the control period specified for each disease. If animals show clinical signs or mortality during the period, the staff of AQS visit the facility for on-site inspection and sampling to detect the *Specified Diseases* by PCR. When the samples turn out to be positive for one of the *Specified Diseases*, all animals are destroyed and the facility is thoroughly disinfected.

On the other hand, according to the Sustainable Aquaculture Production Assurance Act, the manager and employee engaging in aquaculture have to notify the prefectural government in case they recognize or suspect that cultured animals are infected with any of the *Specified Diseases*. The Prefectural Government's Experimental Station that receives the notification conducts preliminary diagnoses for the *Specified Diseases* according to the diagnostic manual issued by MAFF. If the result of the diagnosis is positive, the prefectural government requests a confirmatory diagnosis for the disease to the

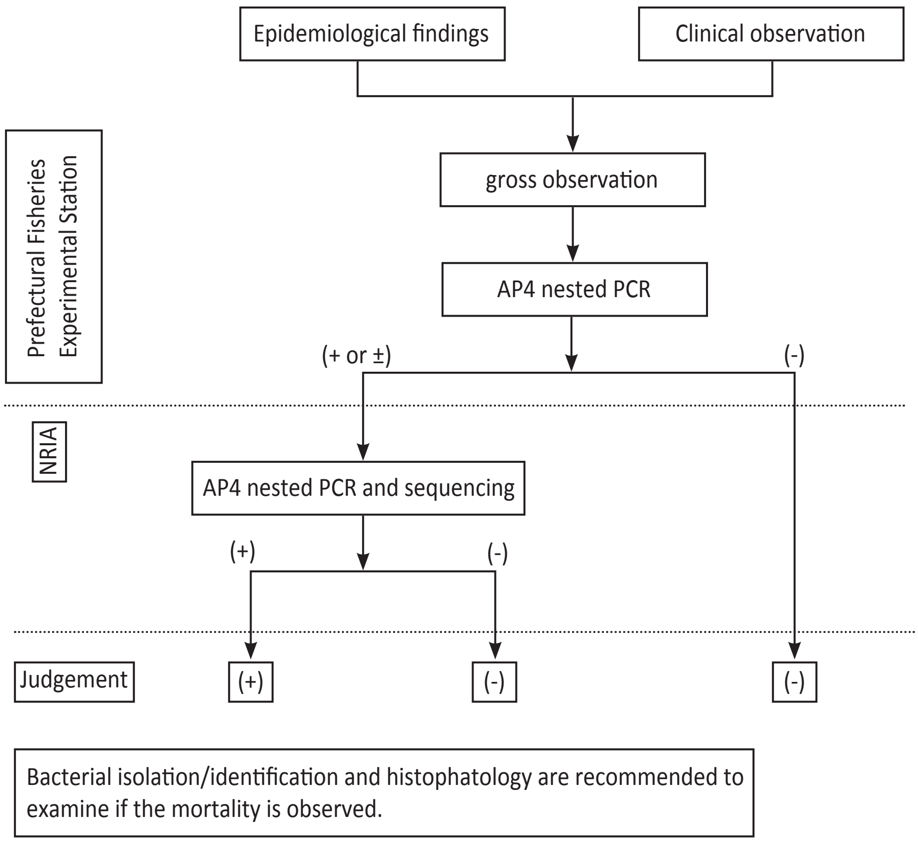

Figure 2. Draft of inspection chart for acute hepatopancreatic necrosis disease (AHPND).

National Research Institute of Aquaculture. Under the instruction of the MAFF, the NRIA should promptly address the request using PCR that is specified in the manual. The PCR adopted for the confirmatory diagnosis is often distinct from the PCR used in the preliminary diagnosis. If the result by the NRIA is also positive for the disease, the prefectural governor can direct the manager or owner of the aquaculture farm to stop transferring the animals from the facility and destroy the animals that are possibly infected with the disease (Figures 2 and 3). In case of outbreaks in the wild environment, the governor can ban fishermen from transferring the susceptible animals from a particular watershed to other waters.

By and large, because the preventive measures highlighted below have been stringently and strictly implemented throughout Japan, occurrence of exotic shrimp diseases including the *Specified Diseases* and those listed in the OIE in Japanese shrimp culture facilities and natural waters has not been so far encountered.

Table 3. List of *Specified Diseases* of shrimp and corresponding diagnostic methods.

Specified Disease	Diagnostic Methods	
	Preliminary test*	Confirmatory test**
Yellow head disease (YHD)	RT-PCR	Nested-PCR
Taura syndrome (TS)	RT-PCR	RT-PCR
Infectious hypodermal and haematopoietic necrosis (IHHN)	PCR	PCR
Tetrahedral baculovirosis	PCR	PCR
Spherical baculovirosis	PCR	PCR
Acute hepatopancreatic necrosis disease (AHPND)	nested-PCR	Nested PCR+sequencing
Necrotising hepatopancreatitis (NHP)	PCR	Real-time PCR
Infectious myonecrosis (IMN)	RT-PCR	Nested-PCR
Covert mortality disease of shrimp (CMD)	RT-PCR	Nested-PCR
Gill-associated virus disease	RT-PCR	Nested-PCR

*Preliminary test is performed at the prefectural experimental stations
**Confirmatory test is performed at the National Research Institute of Aquaculture

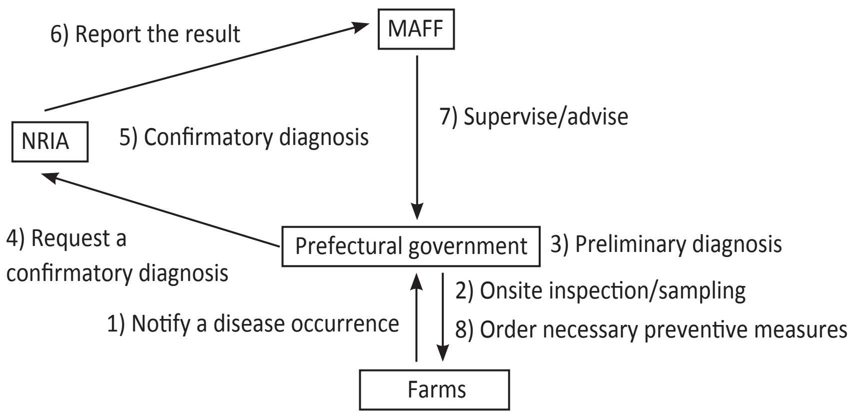

Figure 3. Preventive measures when a *Specified Disease* occurs.

Summary and way forward

Outbreaks of WSD in 1993 due to imported shrimp caused economic losses to the shrimp industry of Japan. Recently, the frequency of WSD occurrence has significantly decreased as a result of WSSV-free broodstock selection, disinfection of fertilized eggs, and UV-treatment of rearing water in juvenile production facilities. On the basis of the lessons learned from cases of exotic infectious disease outbreaks in the past, the government has strengthened regulations to prevent the entry of exotic diseases in the country. Importantly, through stringent and strict implementation of the regulations, Japan has so far been spared from AHPND and other OIE-listed diseases, except WSD. It is therefore evident that the best way to control disease problems in cultivated shrimps and other aquatic animals is to continue embarking on the conduct of scientific researches geared at establishing effective and practical epidemic prevention system for the importation/exportation and domestic movement of live aquatic organisms.

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Status of Aquatic Animal Health Activities in Lao PDR

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Abstract

Lao PDR's National Strategy for Fisheries stipulates the expected outcomes, work plan, and framework aimed at attaining the implementation of current plans and long-term projects up to 2020. Government estimates percent per capita consumption of aquatic animals and aquatic animal products at 15 kg per annum, i.e. accounting for about 40% of the animal protein intake, and targets to increase its per capita availability of fish to 23 kg by the year 2020.

Lao PDR does not have areas for shrimp culture but researches on the migration pattern and reproductive biology of indigenous shrimp species found in the rivers have been undertaken. Inspection of documents for import, transit and export of live aquatic animals at international checkpoints before entry into Lao PDR has been likewise implemented. With regard to importation, permission of import-export (final destination and origin country), certificate of pedigree, and certificate of sanitary quality are being required. In addition, disease-free status (especially those notifiable to the World Organization of Animal Health [OIE]) of imported shrimps and other aquatic organisms is mandatory at international checkpoints before entry into Lao PDR. For shipments suspected to harbor diseases, samples are sent for analysis at the Namxouang Aquaculture Development Center (NADC), Department of Livestock and Fisheries, Ministry of Agriculture and Forestry, Vientiane, Lao PDR.

Introduction

Lao People's Democratic Republic (Lao PDR) is a landlocked country with an area of 236,800 km^2. With no direct access to the sea, capture fisheries are based on water resources ecosystems such as Mekong River and tributaries, large reservoirs, shallow lakes, small natural pools, peat swamps, wetlands, irrigation reservoirs and weirs, rice-fields, small streams and floodplains (Phonvisay, 2013). Tilapia, carp, and catfish are the major species commonly produced in various fish production systems, or caught in reservoirs and natural bodies of water (Theungphachanh, 2004).

Fishing is important not only for the poor, but is essential for all households regardless of socioeconomic group (Garaway, 2005) because fish and other aquatic animals are necessary components of Lao diet. In particular, fish is needed to complement what is lacking in the rice-based diet like essential amino acids, vitamins or minerals. Fish and other aquatic animals also provide additional income and employment through fishery-related activities (Phonvisay, 2013). Moreover, aquatic animals have the highest percentage consumption by weight of animal product (37%) in the diet of surveyed families. This is followed by poultry and livestock at 24% and 23%, respectively (Funge-Smith, 1999).

Aquaculture plays a significant role in sustaining food consumption in the country. When capture fisheries are inaccessible or over-exploited, it serves as an alternative source for fish and other aquatic animals. Farmers are attracted to fish culture since it requires a relatively low entry cost. Ponds can be self-constructed and species like tilapia and carp can be bred in perennial waters rendering farmers to have access to fingerlings. Fish culture is also considered as supplementary activity to small livestock production (Funge-Smith and Dubeau, 2002). Together with capture fisheries, aquaculture plays an essential economic role by contributing 14% to gross domestic product (GDP) of Lao PDR.

Status of fish diseases

Lao PDR has by far not been seriously confronted with issues pertinent to fish diseases compared with other countries in Southeast Asia. However, there were unpublished reports on the occurrence of diseases caused by parasites (*Learnea* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyophthirius multifiliis*, *Trichodina* sp., *Cryptocaryon* sp., *Epistylis* sp., and *Oodinium* sp.) and bacterium (*Edwardsiella tarda*) (Theungphachanh, 2004). Farmers have also reported some ulcerated bodies of catfish or snakeheads whose clinical signs were apparently consistent with epizootic ulcerative syndrome (EUS) (Funge-Smith and Dubeau, 2002). In 1996, diseased snakeheads from Lao PDR were confirmed to be EUS-positive by the Aquatic Animal Health Research Institute (AAHRI), Bangkok, Thailand (Kar, 2016). By and large, comprehensive information on bacterial, fungal, parasitic and viral diseases of freshwater fishes in Lao PDR are not currently available. However, even cases that are deemed as minor diseases, are reported by Lao PDR's focal point to the World Organization of Animal Health (OIE) or Network of Aquaculture Centers in Asia-Pacific (NACA).

Prevention and control of transboundary aquatic animal diseases

Lao PDR imports 24,000 metric tons (MT) per year of seafood products from Thailand and Viet Nam (Phomsouvanh, 2015). Fish seeds are also imported from adjacent countries like Thailand, Viet Nam and China since the demand could not be supplied by in-country production (Funge-Smith, 1999). With regard to importation of live fish and other aquatic animal products, Lao PDR has implemented stringent requirements to prevent the entry and consequential spreading of transboundary aquatic animal diseases into its natural bodies of water. Imported animals and/or products are inspected at the international checkpoint. At present, checking includes detection of diseases, contaminants, and antibiotic residues. Importation requires permit for import-export, certificate of pedigree, and certificate of sanitary quality. The checkpoint ascertains that imported shrimps and other aquatic animals are disease-free especially from infectious diseases listed in the OIE. For shipments suspected to harbor diseases, the Namxouang Aquaculture Development Center (NADC), Department of Livestock and Fisheries, Ministry of Agriculture and Forestry has been assigned to analyze the samples. The laboratory of NADC has the capacity to conduct level II diagnosis, i.e. bacterial, fungal, and parasitic diseases of fish and crustaceans. However, level III diagnostic procedures such as immunological (e.g. enzyme-linked immunosorbent assay [ELISA]) and molecular (e.g. polymerase chain reaction [PCR]) diagnostic tests are not currently available in the center.

Way forward

Lao PDR should take into account the lessons learned by other countries that have been severely affected by transboundary aquatic animal diseases. These information may serve as basis in the formulation of intervention and mitigation strategies aimed at preventing and controlling the inadvertent occurrences of infectious diseases of aquatic organisms as Lao PDR gradually ventures into intensive fish culture to meet the demand of the increasing population. It is therefore imperative that the level of diagnostic capability of Lao PDR's National Fish Health Laboratory has to be continually updated through upgrading of various laboratory equipment and facilities. Importantly, to be adept with the holistic knowledge and skills on fish health management, fish health staff should continually undergo hands-on training on disease diagnosis, quarantine, and surveillance.

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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; Leaño 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

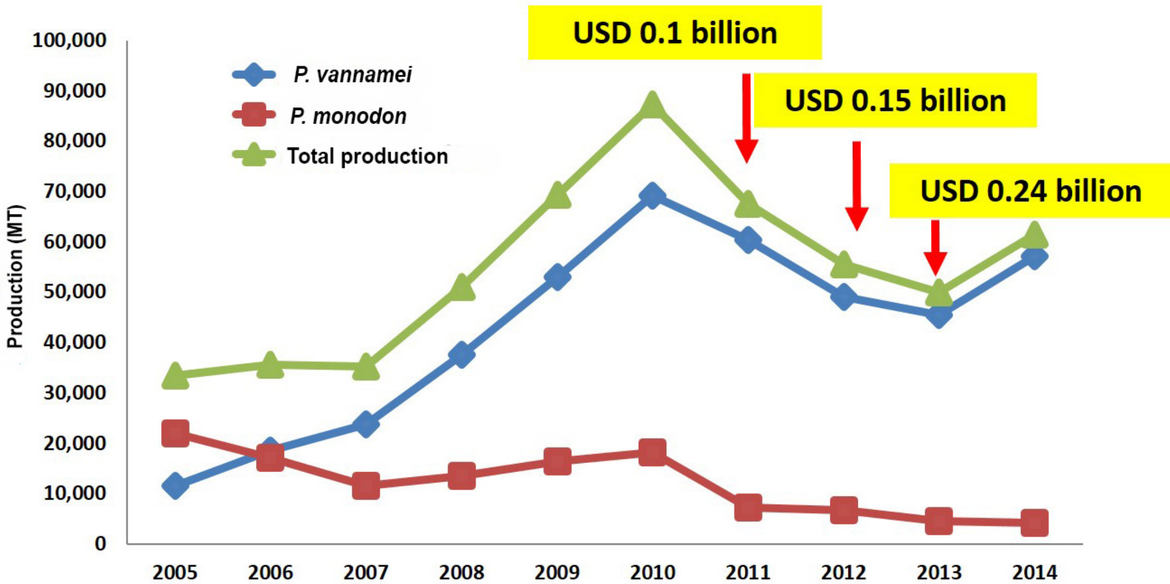

Figure 2. Production of farmed shrimps in Malaysia from 2005 to 2014 and corresponding losses estimated from the reduction of shrimp production.

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 (*P. vannamei*)			Tiger prawn (*P. monodon*)		
	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.

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Status of Shrimp Health Management in Myanmar

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Abstract

Extensive cultivation of the black tiger shrimp (*Penaeus monodon*) started in the 1970's in Myanmar using trap and hold method. *P. monodon* postlarvae (PL) were trapped into the ponds measuring approximately 50 to 100 hectares (ha) during high tide. Because these large ponds have no inputs in terms of pond preparation, eradication of predators, water fertilization, and feeding, production volume during the early years of the shrimp industry in the country provided some lucrative income for the farmers. With this promise, a 3-year project aimed at developing the shrimp culture systems into extensive, extensive plus and semi-intensive was implemented by the Department of Fisheries (DoF), Ministry of Livestock and Fisheries (MLF) in 2010. Unfortunately, in that same year, an outbreak of white spot disease (WSD) caused by white spot syndrome virus (WSSV) occurred in ponds stocked with imported postlarvae and devastated the shrimp industry of the country. Moreover, *P. monodon* samples from Ayeyarwaddy Division (western part of Myanmar) were also found positive for Taura syndrome virus (TSV) and infectious hypodermal and haematopoietic necrosis virus (IHHNV) by polymerase chain reaction (PCR) method in 2010. In addition, yellow head virus (YHV) was also detected in shrimp samples for export in 2014. Fortunately, acute hepatopancreatic necrosis disease (AHPND) has not yet been detected in cultivated shrimps in Myanmar. Because of disease problems, majority of the shrimp farmers have shifted to extensive or traditional shrimp farming. The Aquatic Animal Health and Disease Control Section (AAHDCS) of the DoF formulates the action plans for aquatic animal health and disease control. Thus to keep abreast with the novel techniques used for the detection and management of previously reported and newly emerging diseases of penaeid shrimps, upgrading of laboratory equipment and facilities, and improving the capacity of the departmental personnel on aquatic animal health management are currently being undertaken.

Introduction

Myanmar is endowed with rich and varied marine coastal and inland fishery resources. It has 2,832 km of coastline and total marine fishery areas of 486,000 square kilometers. Moreover, its inland water bodies such as natural lakes, reservoirs, rivers, and ponds cover an area of about 8.2 million hectares (ha). Inland fisheries do not only support the livelihoods of thousands of families but importantly contribute to the fish supplies and national revenue. To increase fish production, culture-based capture fisheries have been practiced in some leasable waters. As a government regulation, the lessee has to release fish fingerlings or juveniles into these identified areas.

Aquaculture has a major role in terms of food security being one of the most important industries contributing to the national economy of the country. Myanmar's fish and shrimp pond areas are approximately 91,653 ha and 92,691 ha, respectively. Pond culture of rohu (*Labeo rohita*), a freshwater fish, is well developed in Myanmar. Notably, production of cultured freshwater fishes does not only supply the country's domestic consumption but likewise other neighboring countries. The local people prefer freshwater fish than marine fish; hence, the Government of Myanmar has laid a policy to target marine fish for the export market.

Shrimp culture in the country started in 1970's using a trap and hold method. Postlarvae (PL) of *Penaeus monodon* were trapped into the ponds during high tide. There were no inputs in terms of pond preparation, eradication of predators, water fertilization, and feeding. As the ponds were usually as large as 50 to 100 ha, the shrimp production provided more than enough income for the shrimp farmers (Thame and Aye, 2005). With this promise, a 3-year project aimed at developing the shrimp culture systems into extensive, extensive plus and semi-intensive was implemented by the Department of Fisheries (DoF), Ministry of Livestock and Fisheries (MLF) in 2010. This was followed by another 3-year project that primarily focused on the development of an intensive culture system (Saw, 2004). However, due to occurrence of diseases, majority of the farmers have shifted back to extensive or traditional shrimp farming system.

The shrimp farming areas in 2013 to 2014 have been estimated at 96,000 ha with the largest areas in Rakhine State (65,324 ha), followed by Ayeyarwady State (24,003 ha), and Yangon State (4,296 ha). Smaller shrimp farming areas could be found in the States of Bago, Kayin, Mon and Thanintharyi (Figure 1). *P. monodon* has been the major species produced, but other species have also been collected from extensive and traditional trap and hold ponds. According

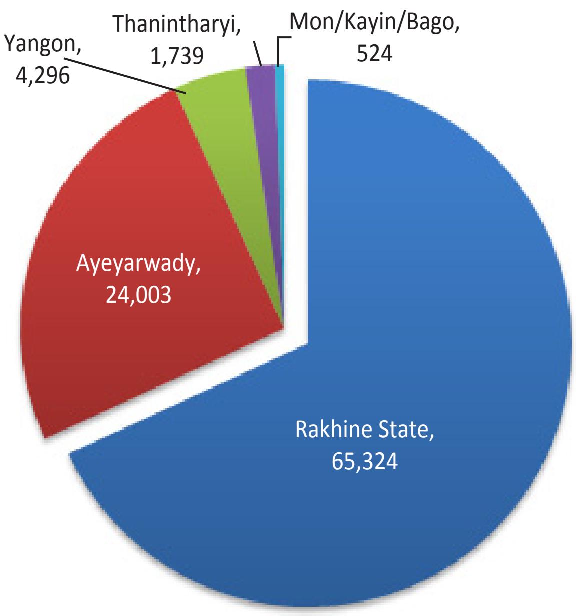

Figure 1. Shrimp farming areas (ha) by region in 2013-2014 (Central Statistical Organization of Myanmar and Department of Fisheries, 2010 and 2013)

to the Food and Agriculture Organization (FAO) of the United Nations, the overall production of *P. monodon* in 2012 was estimated at 52,000 metric tons (MT) giving a production rate of about 550 kg/ha/yr (BOBLME, 2014).

Freshwater prawn (*Macrobrachium rosenbergii*) is also a popular species for aquaculture in Myanmar (Saw, 2004). Captured *M. rosenbergii* was one of the top twenty fishery export items of Myanmar in 2007-2008. It ranked number 13 in the list with a production volume of 1,228.11 MT amounting to 12.28 million U.S. dollars (Fishery and Aquaculture Country Profiles: Myanmar, 2010). Currently, Myanmar has 15 backyard freshwater prawn hatcheries that can produce billions of freshwater prawn seeds (Myanmar Shrimp Association, 2016).

Status of persistent and emerging diseases of cultured shrimp

In 2010, white spot disease (WSD) caused by white spot syndrome virus (WSSV) devastated the shrimp industry of Myanmar. WSD outbreak occurred in intensive shrimp ponds stocked with imported postlarvae. Consequently, the disease spread to other ponds through horizontal transmission (Saw, 2004). WSSV was also detected by PCR method in shrimp samples for export in 2014 (NACA and FAO, 2015).

Taura syndrome (TS) and infectious hypodermal and haematopoietic necrosis (IHHN) caused by Taura syndrome virus (TSV) and infectious hypodermal and haematopoietic necrosis virus (IHNNV), respectively, have been officially reported in Myanmar (AGDAFF-NACA, 2007). In January 2010, 40 samples of *P. monodon* from Ayeyarwaddy Division (western part of Myanmar) were submitted to DoF for PCR analysis. Ten percent of the samples examined were found positive for TSV while only 2.5% were positive for IHHNV (NACA and FAO, 2010). Yellow head virus (YHV) was also detected in shrimp samples for export in 2014 (NACA and FAO, 2015). Fortunately, acute hepatopancreatic necrosis disease (AHPND) has not been reported yet in Myanmar.

In February 2013, another case of serious mortality was also observed in hatchery-reared freshwater prawn (*M. rosenbergii*) PL in two townships (Yangon Region). At that time, 50 million *M. rosenbergii* PL 5-6 were affected. As a consequence, farmers lost about USD 2 million. Because the broodstocks were unhealthy, the larvae and PL were weak. The infection started in the summer time when the temperature was high and the water quality was also poor. Affected freshwater prawn exhibited symptoms including change in the coloration of the dorsal side and eyes and pale body color that eventually turned purple. None of the World Organization for Aquatic Animal Health (OIE)-listed pathogens was detected in the fixed diseased samples sent to the Laboratory of Dr. D. Lightner in Arizona State University, USA. Because farmers speculated that the etiology of the disease was bacterial, they used antibiotics to treat the disease. However, this intervention failed to control the disease; hence, the DoF advised farmers to maintain good water quality, reduce stress and use nutritionally adequate feeds to prevent further mortalities.

Laboratory and diagnostic capacity

The DoF is the responsible agency and competent authority for the management and sustainability of the fishery development in Myanmar. It enhances food security by increasing fish production for domestic consumption and export. Practical and effective strategies aimed at preventing and controlling the occurrence of diseases in hatcheries and grow-out facilities through proper aquatic animal health management have been identified as an important priority area of the DoF to make production of different aquaculture species sustainable. To address this need, the DoF established the Aquatic Animal Health and Disease Control Section (AAHDCS) under the Aquaculture Division. The main laboratory of the AAHDCS is located in Yangon (Yangon Region). The other AAHDCS laboratory is located in Nyaung Don (Ayeyawaddy Region). Figure 2 shows the organizational chart of the AAHDCS of Myanmar.

The AAHDCS has been conducting surveillance using Level-1 disease diagnosis. The activities covered in the surveillance include the following: (a) report from the township fisheries officers; (b) report from the farmers directly to the aquatic animal health section; (c) occasional field visit of township fisheries officers to farm sites; and (d) recording of reports on disease occurrences. The AAHDCS has also the capability to conduct Level-II diagnosis, i.e. through histopathology and microbiology; however, upgrading of equipment and training of staff have to be undertaken. Notably, the AAHDCS laboratory has the capability to conduct Level-III diagnosis such as the use of PCR method to detect shrimp viral diseases including WSD, IHHN, TS and YHD. In addition, the AAHDCS laboratory plans to establish the diagnostic methods for

Figure 2. Organizational chart of Aquatic Animal Health and Disease Control Section of Myanmar.

AHPND following the suggested methods in the OIE Manual.

Disease prevention and control strategies

Because of shrimp diseases, annual shrimp production over the past years has decreased. To mitigate losses, it is crucial to implement precautionary measures to prevent and control the occurrence of diseases and pathogen population in the hatcheries and grow-out ponds. It is also important to prevent water quality from deteriorating and to strengthen the natural resistance of the shrimp stocks.

In Myanmar, the AAHDCS formulates action plans for aquatic animal health and disease control which include: (a) collecting shrimp disease information by active and passive reporting systems. Mobile teams regularly visit premises before export and provide necessary instructions. Also, training on aquatic animal health management is provided to shrimp farmers and students; (b) issuance of health certificate after the animal has been examined to be healthy and free from any clinical sign of disease; (c) checking of transboundary aquatic animal diseases in live aquatic animals for import or export being performed at Yangon International Airport; (d) dissemination of pamphlets for aquatic animal disease information and prevention of aquatic animal diseases; and (f) regular submission of quarterly report on aquatic animal diseases to the Network of Aquaculture Centres in Asia-Pacific (NACA) and OIE. The AAHDCS has prepared a *Handbook of Aquatic Animal Health Management* for fish farmers and students. The manual encompasses topics on aquatic animals susceptible to a particular disease, stage(s) affected, key clinical signs of the disease and concomitant diagnostic methods among others.

Way forward

With the expansion of farming areas for aquaculture, occurrence of diseases will be inevitable; hence, adherence to good aquaculture practices should be observed. Since the AAHDCS of the DoF plays a crucial role in the formulation of the action plans for aquatic animal health and disease control, upgrading of laboratory equipment and facilities, and improving the capacity of the departmental personnel on aquatic animal health management are currently being undertaken to keep abreast with the novel techniques used for the detection and management of previously reported and newly emerging transboundary diseases of penaeid shrimps and other economically important aquatic organisms.

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Table 1. Levels of disease diagnosis and corresponding activities at the Aquatic Animal Health and Disease Control Section of Myanmar.

Level	Site	Activities
I	Field	Surveillance, observation of animal and the environment, clinical examination and extension service
II	Laboratory	Diagnostics (parasitology, bacteriology, mycology, histopathology) and extension service
II	Laboratory	Diagnostics (PCR for WSSV, YHV, TSV using the IQ 2000TMKit) and extension service

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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

Continued from previous page

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.

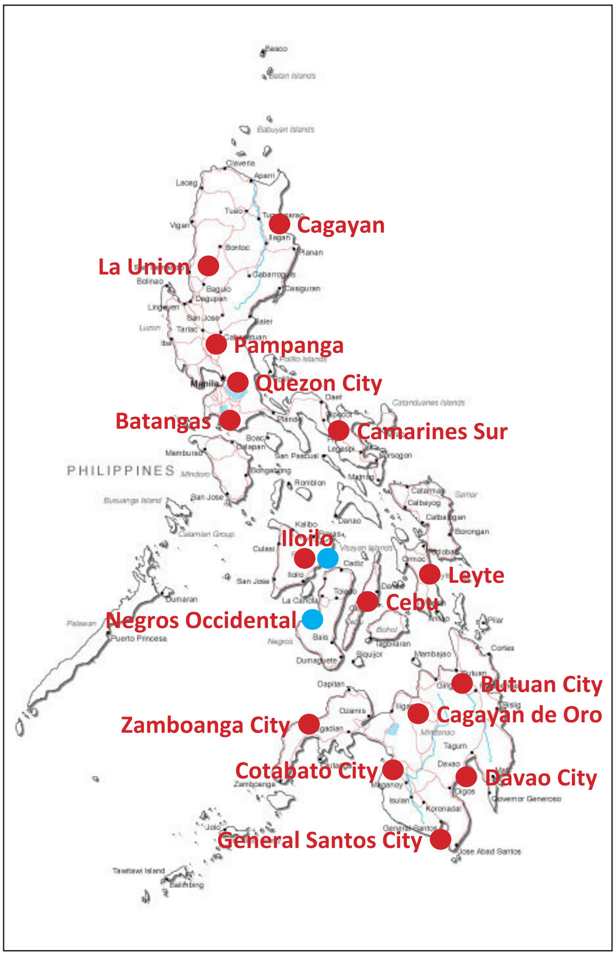

Figure 1. Distribution of BFAR (●) and BFAR-recognized (●) Diagnostic Laboratories for shrimp disease detection in the Philippines.

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.

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Status of Transboundary Diseases of Penaeid Shrimps in Singapore

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Abstract

Singapore has a small shrimp farming industry with approximately 54 metric tons (MT) of shrimps produced in one year from both land and coastal farms. There is also one shrimp farm producing broodstock for export. Singapore has the capability to diagnose acute hepatopancreatic necrosis disease (AHPND) through histopathology and the isolation of its causal agent which is *Vibrio parahaemolyticus*. The polymerase chain reaction (PCR) method for AHPND detection in shrimp is currently being undertaken to further strengthen its laboratory and diagnostic capacity. Notably, Singapore is still AHPND-free. On the contrary, white spot disease caused by white spot syndrome virus (WSSV) is a disease of concern as it affects the trade for ornamental crustaceans. Singapore has an active surveillance program for WSSV and other transboundary pathogens of penaeid shrimps. Positive detections would be followed by movement controls and stamping out protocols.

Introduction

Singapore is a small island state with a total population of over 5 million in 2014 (Yearbook of Statistics Singapore, 2015). With small farming land and limited fishing grounds, it imports over 90% of food consumed in the country (Agri-Food and Veterinary Authority of Singapore, 2015). Local farms have little contribution to food supply. For example, out of the 100,000 metric tons (MT) per year of fish consumed, only 5% has been attributed to domestic food fish aquaculture (Tan, 2015). Majority of the fish produced comes from coastal farming, i.e. in floating net cages, along the northern coast of Singapore. Sea bass, pompano, groupers, mullets, and milkfish are the marine food fish species popularly cultured in the country. There are also a few land-based fish farms culturing tilapia, marble goby and snakehead (Heng, 2015). The country is also known as the ornamental fish capital of the world. It exports about 2.4 million pieces of koi annually to the United Kingdom, Germany, United States and Malaysia (Ling *et al.*, 2005). Farming of penaeid shrimps in Singapore started in the 1980s with the dominance of black tiger (*Penaeus monodon*) and banana (*P. merguiensis*) shrimps. Cultivation of specific-pathogen-free (SPF) *P. stylirostris* and *P. vannamei* started in 2001 and 2002, respectively. Shrimp production reached 60 MT in early 2000 and increased to 114 MT in 2001 and 115 MT in 2002. However, due to urbanization and occurrence of diseases, shrimp production dropped to 48 MT and 46 MT in 2003 and 2004, respectively (Choi and Serena, 2005). Currently, Singapore has a small shrimp farming industry, with approximately 54 MT of shrimps produced in one year, from both land-based and coastal farms. There is also one shrimp broodstock farm producing broodstock for export.

The increase in international trade of ornamental fish poses challenge to Singapore in terms of its vigilance against exotic aquatic animal diseases (Ling *et al.*, 2005). Furthermore, reliance on imported seafood opens the risk for transboundary aquatic animal diseases. The establishment of the Agri-Food and Veterinary Authority (AVA) has over the past years addressed these challenges and issues. AVA is the national authority for aquaculture development in Singapore and manages aquaculture farms through the issuance of fish farming licenses (Heng, 2015). It also carries out surveillance for significant pathogens and conduct regular surveys and inspections of fish farms and exporters' premises (Ling *et al.*, 2005). AVA consists of five Departments. Aquatic animal health diagnostic services are provided by one of the Departments – the Animal Health Laboratory Department (AHLD).

Acute hepatopancreatic necrosis disease (AHPND) has recently gained remarkable attention among major shrimp producing countries in Asia. It has significantly impacted the shrimp industry of China, Viet Nam, Malaysia, Thailand and the Philippines (Tran *et al.*, 2013; Joshi *et al.*, 2014; Dabu *et al.*, 2015; de la Peña *et al.*, 2015). AHPND has also been implicated in serious economic losses of cultured penaeids in Mexico (Nunan *et al.*, 2014). There is one shrimp broodstock farm in Singapore, producing broodstock for export. The farm's shrimp health monitoring programme currently in place includes samples being submitted every week to test for EMS/AHPND. Fortunately, AHPND in cultivated shrimp has not been detected yet in Singapore. On the contrary, white spot disease (WSD) caused by white spot syndrome virus (WSSV) has, over the past several years, been a disease of economic importance to Singapore as it affects the export trade of ornamental crustaceans. WSSV is also a disease notifiable to the national government of Singapore.

There is an active surveillance programme for WSSV. Other than inspections for clinical signs, samples are collected from both imported and local ornamental crustaceans for real-time PCR testing. For shrimps showing clinical signs, histopathology would also be done. Positive detections would warrant movement controls to be imposed, while further investigations would be carried out to determine the source of infection. Stamping out of the infected batch, together with other in-contact susceptible species, would be conducted. The farm would have to undergo cleaning and disinfection of the tanks/ponds before being allowed to restock. Further testing may be conducted to ascertain that the disease has been eradicated.

Status of persistent and emerging diseases of cultured shrimp

The shrimp industry in Singapore incurred significant losses in the 1990s due to outbreaks of diseases caused by yellow head virus (YHV) and white spot syndrome virus (WSSV) (Choi and Serena, 2005). Based on the annual report of AVA (2009/2010 and 2013/2014) and Ornamental Fish Newsletter (Agri-Food and Veterinary Authority of Singapore, 2010), WSSV was detected in prawns from local land-based farms and in a batch of crayfish submitted by a research institute. Over the past three years, WSSV has been detected in imported shrimps (June 2013), imported lobsters (December 2013), and imported ornamental crayfish (December 2014 and September 2015).

Laboratory and diagnostic capacity

The Animal Health Laboratory Services of AVA aims to maintain Singapore's animal disease–free status in order to facilitate the country's international trade, protect the health of the local animal populations, and indirectly safeguard human health (Agri-Food and Veterinary Authority of Singapore, 2016). It is divided into four sections namely: (a) Aquatic Animal Health, (b) Bacteriology, (c) Virology and (d) Veterinary Pathology. The laboratory was awarded with ISO/IEC 17025 SAC SINGLAS Accreditation in 2004 with more than 108 tests accredited. The Aquatic Animal Health section deals with all aquatic animal diseases. Currently, it has three veterinarians, three scientists and two laboratory technicians. In terms of training, the institute sent two veterinarians for a short shrimp pathology course at the University of Arizona, USA. AVA performs diagnostic services such as post-mortem examination, wet mount microscopy, histopathology, bacterial and fungal culture, virus isolation and molecular detections of pathogens. The section also conducts services like pre-export testing of ornamental fish, basic health monitoring of aquaculture establishments,

Table 1. List of shrimp pathogens and diagnostic methods employed at the Animal Health Laboratory Services of Agri-Food and Veterinary Authority (AVA) of Singapore.

Shrimp Pathogen	Sample Type	Diagnostic Method
Taura syndrome virus (TSV)	Pleopod or whole postlarvae (PL)	Histopathology; Real-time PCR
White spot syndrome virus (WSSV)	Pleopod or whole PL	Histopathology; Real-time PCR
Yellow head disease virus (YHDV)	Pleopod or whole PL	Histopathology; Real-time PCR
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Pleopod or whole PL	Histopathology; Real-time PCR
Baculovirus penaei (BP)	Whole PL or shrimp	Wet mount examination; Histopathology; Real-time PCR
MBV - Spherical baculovirus		
(*Penaeus monodon*-type baculovirus)	Whole PL or shrimp	Wet mount examination; Histopathology
Infectious myonecrosis virus (IMNV)	Whole PL or shrimp	Histopathology; Real-time PCR
Obligate intracellular rickettsial-like organism causing necrotising hepatopancreatitis (NHP)	Whole shrimp	Histopathology; Real-time PCR
Vibrio parahaemolyticus causing acute hepatopancreatic necrosis disease (VP_{AHPND})	Whole shrimp or hepatopancreas	Culture and biochemical tests for *Vibrio parahaemolyticus* (VP_{AHPND}); Histopathology
Enterocytozoon hepatopenaei (EHP)	Whole shrimp or hepatopancreas	Histopathology

Table 2. List of molecular methods, equipment, and primers used for the detection of infectious shrimp pathogens at the Animal Health Laboratory Services of Agri-Food and Veterinary Authority (AVA) of Singapore.

Shrimp Pathogen	Method, equipment, and primers used
Taura syndrome virus (TSV)	TaqMan Real Time; ABI AgPath ID RT-PCR kit Primers: 1004F/1075R, TSV-P1 probe
White spot syndrome virus (WSSV)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1011F/1079R
Yellow head disease (YHD)	ABI AgPath ID RT-PCR kit; Primers: 141F/206R; YHV TaqMan
Infectious hypodermal and haematopoietic necrosis (IHHNV)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1608F/1688R
Infectious myonecrosis virus (IMNV)	ABI TaqMan One-Step RTPCR Master Mix; Primers: new412F/545R, IMNVp1
Necrotising hepatopancreatitis (NHP)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1300F/1366R
Nucleic acid/ Enzyme	**Detection method**
RNA extraction	MPLC Total Nucleic Acid Kit (ROCHE)
DNA extraction	MPLC Total Nucleic Acid Kit (ROCHE) & QIAGEN QIAamp DNA Mini Kit (for NHP real-time PCR)
RT-PCR enzyme	ABI AgPath ID RT-PCR kit & ABI TaqMan One-Step RTPCR Master Mix
PCR enzyme	Maxima Probe/Rox qPCR Master Mix

surveillance programs for marine food fish diseases and on-site consultation. Animal Health Laboratory Services of AVA has the capability to diagnose economically important viral diseases of penaeid shrimps (Table 1) using different molecular methods (Table 2). In addition, it has also the capability to diagnose shrimps suffering from AHPND through histopathology and isolation of its causal agent which is *V. parahaemolyticus* (VP_{AHPND}) (Table 1). The establishment of the polymerase chain reaction (PCR) assay for AHPND is currently being undertaken to further strengthen AVA's diagnostic capacity.

Disease prevention and control strategies

Singapore has an active surveillance program for WSD. Active surveillance likewise includes World Organization for Animal Health (OIE)-notifiable and emerging diseases of penaeid shrimps and fish (Agri-Food and Veterinary Authority of Singapore, 2011). The frequency of sampling for WSD is being conducted twice a year per farm establishment. The data collected from the surveillance have by far been useful in identifying disease trends and risks beneficial in the review of AVA's surveillance programs. AVA has extended the national surveillance to breeders and non-export farms since early 2009. This move has facilitated the early detection of aquatic diseases thereby allowing AVA to certify disease-free status of the farms at the national level and opened up accessibility to major markets like the European community (Agri-Food and Veterinary Authority of Singapore Annual Report 2009/2010).

The Ornamental Fish Newsletter (Agri-Food and Veterinary Authority of Singapore, 2010) cited some of the recent activities for WSD. First, WSD is notifiable to AVA under the Animals and Birds (Disease) Notification. Based on section 30 of Animals and Birds Act, offense will be imposed to a person who does not report cases of animal infected with or reasonably suspected to be infected with the disease(s). He/she shall be liable on conviction to a fine not exceeding USD 10,000 or imprisonment not exceeding 12 months or to both. Second, submission of a health certificate issued by the competent authorities of exporting countries is required in order to import ornamental aquatic animals. The requirement is applicable to ornamental species which are susceptible to seven diseases including WSD. Third, in order to increase awareness, AVA has distributed disease cards on WSD and other diseases to licensed importers and exporters. The card includes clinical signs to look out for and pictures of diseased animals. Lastly, adoption of good biosecurity measures and sanitary practices in the premises is highly recommended.

For disease control, warrant movement controls shall be imposed in cases where pathogen detection in the animal becomes positive. This step is followed by stamping out of the infected batch, together with other in-contact susceptible species. The farm has to undergo thorough cleaning and disinfection of tanks and/or ponds before being allowed to restock. Further testing will be likewise conducted to ascertain that the disease has been eradicated.

In addition, follow up actions will also be undertaken such that if detection is in the stock that is outside the quarantine area, a 2-year suspension to sell susceptible species to countries requiring the disease freedom will be strictly observed. The aforementioned premises will also undergo a minimum of twice a year sampling for that particular disease and that, suspension will only be lifted after attaining negative results for two successive years of sampling.

Way forward

In conjunction with AVA's research and development program, early detection of infectious transboundary shrimp pathogens will still be the main focus to maintain Singapore's animal disease-free status thereby facilitating the country's international trade, protecting the health of local animal populations, and more importantly, safeguarding human health. In addition, the Animal Health Laboratory Services of AVA will continually conduct its surveillance program on important shrimp pathogens especially those notifiable to the OIE.

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Current Status and Impact of Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Disease (AHPND) and Hepatopancreatic Microsporidiosis (HPM) Outbreaks on Thailand's Shrimp Farming

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Abstract

Outbreak of early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) in whiteleg shrimp (*Penaeus vannamei*) and black tiger shrimp (*P. monodon*) was first documented in August 2011 in Eastern Thailand. The disease subsequently spread to almost all shrimp production areas in 2012 until the early part of 2016. These episodes of AHPND outbreaks consequently impacted the shrimp industry as evidenced by significant reduction in the production of farmed shrimps, shortage of raw materials for the shrimp export industry, and reduction of global shrimp supply from Thailand. Following the discovery of *Vibrio parahaemolyticus* as the causal agent of AHPND, PCR techniques subsequently became available for the rapid and accurate detection of AHPND in cultivated shrimps. The Department of Fisheries (DOF) consequently included AHPND in the National Surveillance Program focusing on the investigation of risk factors responsible for the outbreak and concomitant spread of the disease. As a result, the quality of broodstock and postlarvae (PL) and as well as farm management practices, i.e. pond bottom and water preparation, stocking density, feeds and feeding practices, and water quality fluctuations were identified as key risk factors associated with AHPND outbreaks. By and large, the DOF has undertaken mitigating measures to control and prevent further outbreaks of AHPND including the improvement of sanitation in marine shrimp broodstock and PL hatcheries, quality evaluation and disease screening of broodstocks and PL, detection of pathogens in soil and water samples, and acquisition of new broodstocks for improved genetic diversity. To date, Thailand's shrimp industry has gradually recovered from the devastating effects of AHPND since 2015.

Heptopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP), a spore-forming microsporidian, is another emerging disease of cultured penaeids in Thailand. EHP was first documented in farmed *P. monodon* in 2004, however, its impact was not clearly evaluated at that time. EHP was again observed in *P. vannamei* in 2014 at the same period of AHPND outbreak in Thailand. In the field, EHP could be transmitted horizontally through feeding of the EHP-contaminated feed and feces from infected shrimp. Samples collected from numerous shrimp farms showed that EHP was heavily present in both ponds with successful and failed crops indicating that EHP infection in shrimp may not be a significant contributing factor to a failed production run. *In vitro* challenge likewise showed that there was no correlation between EHP and white feces syndrome. However, EHP infection at significantly high levels could affect shrimp growth. One of the mitigating measures to control EHP infection in cultured shrimp is the reduction of contamination in hatcheries and grow-out facilities.

Despite the negative impacts of AHPND and HPM on the shrimp industry of Thailand, all parties of the shrimp sector have been working in concert to attain the projected annual shrimp production volume of approximately 300,000 metric tons (MT) in 2016.

Introduction

Thailand is one of the major marine shrimp-producing countries in Southeast Asia exporting about 85% of its annual shrimp production to many regions of the world including USA, EU, Japan, and Canada, among others. Since 1987, extensive farming of the black tiger shrimp (*Penaeus monodon*) along the 2,614 km coastline of Thailand gradually shifted to the intensive system (Tookwinas, 1999). Shrimp production has increased to more than 13 folds, i.e. from 23,566 metric tons (MT) in 1988 to about 309,862 MT in 2000 (Figure 1). The significant increase in shrimp production has been primarily attributed to the tropical climate that creates an ideal environment for intensive marine shrimp farming (Szuster, 2006).

However, outbreaks of yellow head disease (YHD) caused by yellow head virus (YHV) and white spot disease (YSD) caused by white spot syndrome virus (WSSV) since 1992 and 1994, respectively, have negatively impacted the growth and sustainability of the Thailand's shrimp industry (Booyaratpalin, 1999). In 2002, another devastating disease named monodon slow growth syndrome (MSGS) emerged and affected shrimp growing areas throughout Thailand and significantly reduced the annual shrimp production volume by approximately 36% (Flegel, 2008). The etiology of this disease was not determined but laboratory trials suggested the involvement of a filterable infectious agent (Withyachumnarnkul, *et al.*, 2004). Because of the significant economic losses brought about by MSGS to the *P. monodon* industry in Thailand, majority of the shrimp growers eventually shifted to *P. vannamei* culture since 2004.

Whiteleg shrimp was introduced to the shrimp farms in Thailand in 2003 after the significant drop of *P. monodon* production. *P. vannamei* proved to be a good candidate species for intensive shrimp farming in Thailand as evidenced by a remarkable increase in the production volume reaching nearly 600,000 MT from 2009 to 2010 (Figure 1). However, just like the other major shrimp-producing countries in the region, shrimp production in Thailand has been hampered by outbreaks of acute hepatopancreatic necrosis disease (AHPND) since 2012. Recently, an emerging infectious disease caused by the microsporidian *Enterocytozoon hepatopenaei* (EHP) has extensively spread among cultured penaeids in Thailand.

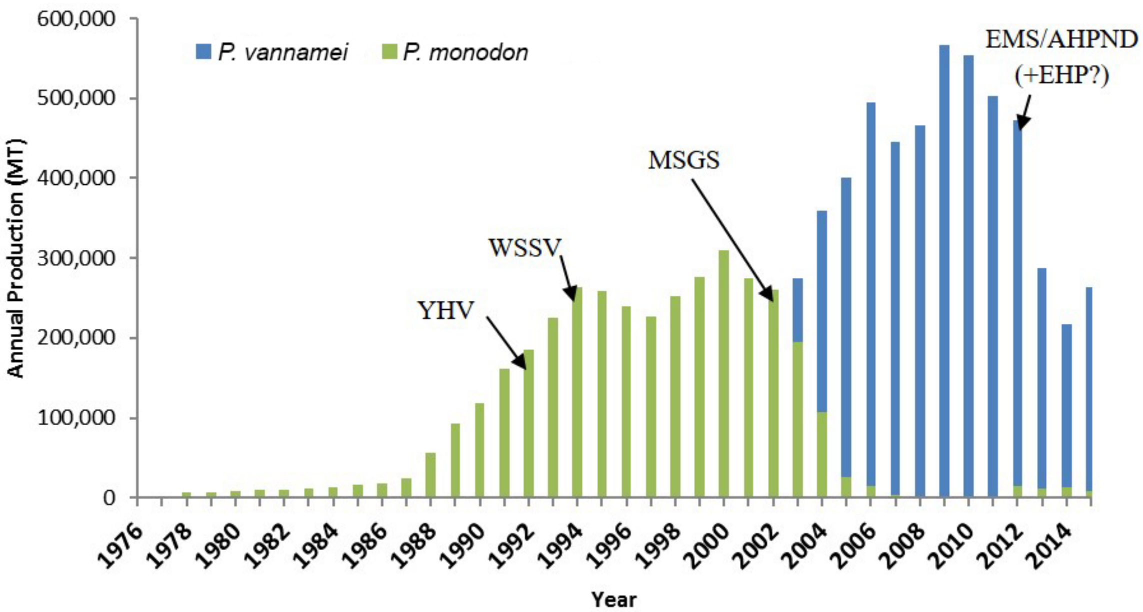

Figure 1. Annual shrimp production in Thailand from 1978 to 2015 illustrating the transition period of *Penaeus vannamei*'s dominance over *P. monodon* in terms of production volume. Black arrows point to the production years where major disease outbreaks occurred in cultured shrimps.

Status of acute hepatopancreatic necrosis disease (AHPND)

AHPND, formerly called EMS, is an infectious bacterial disease that caused significant economic losses among cultivated shrimps in Thailand. The first outbreak of AHPND began in China in 2009 and subsequently spread to Viet Nam and Malaysia in 2010 and 2011, respectively (Tran *et al.*, 2013). In Thailand, the first reported outbreak of AHPND was in August of 2011 in a pond located in the eastern province of Thailand (Chucherd, 2013).

The Aquaculture Pathology Laboratory of the University of Arizona isolated *Vibrio parahaemolyticus* in pure culture from the hepatopancreas of shrimp infected with AHPND in 2013 (Tran *et al.*, 2013). Subsequent immersion challenge of healthy shrimps with *V. parahaemolyticus* strain isolated from the hepatopancreas of AHPND-infected shrimp led to the development of clinical signs similar to what have been previously observed in naturally-infected shrimp, indicating that indeed the *V. parahaemolyticus* strain isolated from the hepatopancreas of the diseased shrimp is the etiologic agent of AHPND (Tran *et al.*, 2013). Molecular diagnostic tests were then developed for the rapid detection of AHPND-causing *V. parahaemolyticus* (VP_{AHPND}) strains using polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) methods (Flegel and Lo, 2013, Tinwongger *et al.*, 2014; Kowai *et al.*, 2016). The Department of Fisheries (DOF) of Thailand has an ongoing collaboration with Tokyo University of Marine Science and Technology (TUMSAT) on the use of a Multiplex PCR technique to investigate and differentiate the presence of typical *V. parahaemolyticus* and VP_{AHPND} strains in shrimp samples (Tinwongger *et al.*, 2014). The causative bacterium has a specific plasmid that is responsible for the production of PirA and PirB toxins, which have been proven to be the direct cause of acute necrosis in the shrimp's hepatopancreas. The gross clinical signs exhibited by shrimps with AHPND include soft or loose shell, pale coloration and significant atrophy of hepatopancreas (Figure 2). Moreover, the infected shrimps manifested

Figure 2. Photograph of a normal (A) and acute hepatopancreatic necrosis disease (AHPND)-infected shrimp exhibiting an empty gut (yellow arrow) (B). (Photo courtesy of the Department of Fisheries, Thailand)

Table 1. PCR detection of acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus* (VP_{AHPND}) plasmid and toxin genes in the different stages of cultured whiteleg shrimps.

Types of sample	PCR detection of VP_{AHPND} plasmid: No. of sample tested	PCR detection of VP_{AHPND} plasmid: Percentage (%) of positive sample tested	VP_{AHPND} toxin gene: No. of sample tested	VP_{AHPND} toxin gene: Percentage (%) of positive sample tested
Broodstock	73	0	73	0
Broodstock feces	27	19	27	0
Nauplii	53	0	53	2
Post larvae	2,174	11	1,363	5
Juvenile	1,490	28	1,261	18
Farm water	3,116	29	2,166	22
Hatchery water	542	19	23	0
Farm sediment	1,614	36	1,054	17

Table 2. Acute hepatopancreatic necrosis disease (AHPND)-positive whiteleg shrimp (*Penaeus vannamei*) postlarvae produced from the different shrimp farming areas in Thailand determined by PCR method.

Location of hatchery	AHPND detection by PCR method in *Penaeus vannamei* postlarvae		
	No. of samples tested	No. of positive samples tested	Percentage (%) of positive samples tested
Eastern provinces	120	9	7.5
Southern provinces	80	11	13.75
Total	200	20	10

Table 3. Estimated volume of annual shrimp production losses due to acute hepatopancreatic necrosis disease (AHPND) in Thailand from 2012 to 2015.

Year	Shrimp Production (MT)			Estimated annual production losses (MT)
	P. monodon	*P. vannamei*	Total	
2011	1,469	500,719	502,188	-
2012	15,219	458,012	473,231	28,957
2013	12,124	274,755	286,879	215,309
2014	13,053	204,385	217,438	284,750
2015	7,828	255,294	263,122	239,065

slow growth, empty guts, swim sluggishly or spirally along the dikes followed by mass mortality within 7 to 35 days after stocking.

All stages of shrimp can be infected by VP_{AHPND}. The target species are both the whiteleg and black tiger shrimps with the former seemingly the more susceptible species to AHPND. PCR detection of VP_{AHPND} plasmids and toxin genes in whiteleg shrimp juveniles collected from farms affected by AHPND outbreaks in 2015 showed the highest number of samples positive for plasmids (28%) and toxin gene (18%) (Table 1). PCR detection of VP_{AHPND} in postlarvae samples collected from hatcheries located in the Eastern and Southern provinces of Thailand from 2013 to 2014 have prevalence rates of ca. 7.5% and 13.75%, respectively (Table 2). In addition, as shown in Table 1, VP_{AHPND} could be found in the pond and hatchery water, as well as farm sediment with prevalence rates ranging from 19-36%.

Outbreaks of AHPND which commenced in 2012 have over the past 4 years continued to cause significant losses among cultivated shrimps produced in Thailand. Notably, the cumulative volume of production losses from 2012 to 2015 totaled to ca. 768,081 MT. As shown in Table 3, the annual shrimp production volume increased from 217,437 MT in 2014 to 263,122.90 MT in 2015, indicating that the shrimp industry of Thailand has apparently recovered from the negative impacts of AHPND.

Prevention and control AHPND

Effective mitigating measures geared at curbing AHPND outbreaks in shrimp farms include strict adherence to biosecurity and good shrimp aquaculture practices. In Thailand, the following *Guidelines for good shrimp farming practices* was issued by the DOF in 2014:

- Appropriate pond preparation to remove excess organic matter from the pond bottom;
- Intensification of farm biosecurity and water treatment;
- Increase in the number of reservoirs and water filtration systems to eliminate fish and disease carriers;
- Monitoring of postlarvae quality and health condition;
- Stocking of shrimp at appropriate density according to farm capacity;
- Maintenance of good water quality in

order to prevent anoxic condition of the pond bottom, phytoplankton blooms, and water quality fluctuations. Oxygen should be maintained at acceptable levels to promote good growth;

- If necessary, useful probiotics should be applied to pond water during the course of shrimp culture. The use of probiotics (e.g. *Bacillus subtilis*) has been promoted by the Thai government since 2013 for maintaining good water quality and shrimp health;
- Monitoring of cultivated shrimps for the presence of pathogens and removal of susceptible species;
- If cultured shrimps exhibit clinical signs of AHPND, laboratory analysis should be conducted to confirm the case. In case an outbreak of AHPND occurs in a grow-out pond, termination of culture is necessitated, and thorough chlorination of the pond water should be executed.

Status of hepatopancreatic microsporidiosis (HPM)

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (EHP), a microsporidian first discovered in *P. monodon* in Thailand in 2004 (Chayaburakul *et al.*, 2004) and later described in detail and named (Tourtip, 2005; Tourtip *et al.*, 2009). EHP infects only the tubule epithelial cells of the hepatopancreatic (HP) tissue of shrimp. EHP was also later found to infect cultured *P. vannamei* in Thailand (Tourtip *et al.*, 2009). Through the wet mount method, EHP spores could be observed in the hepatopancreas of infected shrimps stained with Phloxine B solution (2% w/v) by light microscopy at 100x magnification (Figure 3.) PCR and *in situ* hybridization methods for EHP detection were initially described by Tourtip, *et al.* (2009). The PCR detection method was later improved using nested PCR primers synthesized based on nucleotide sequences of the small subunit ribosomal RNA (ssu rRNA) gene with PCR products of 779 bp and 176 bp, respectively (Figure 4) (Tangprasittipap *et al.*, 2013). More recently, alternative *in situ* and PCR detection (Tang *et al.*, 2015), real-time PCR (Liu *et al.*, 2014) and LAMP-nanogold methods (Suebsing *et al.*, 2013) have also been described. In general, EHP reduces the growth rate and increases the size variation of cultured penaeids. The degree of EHP infection was found to be primarily influenced by the number of spores in the hepatopancreas of HPM-infected shrimp. Additionally, the higher the number of spores present in the shrimp's hepatopancreas, the greater is its impact in stunting shrimp's growth.

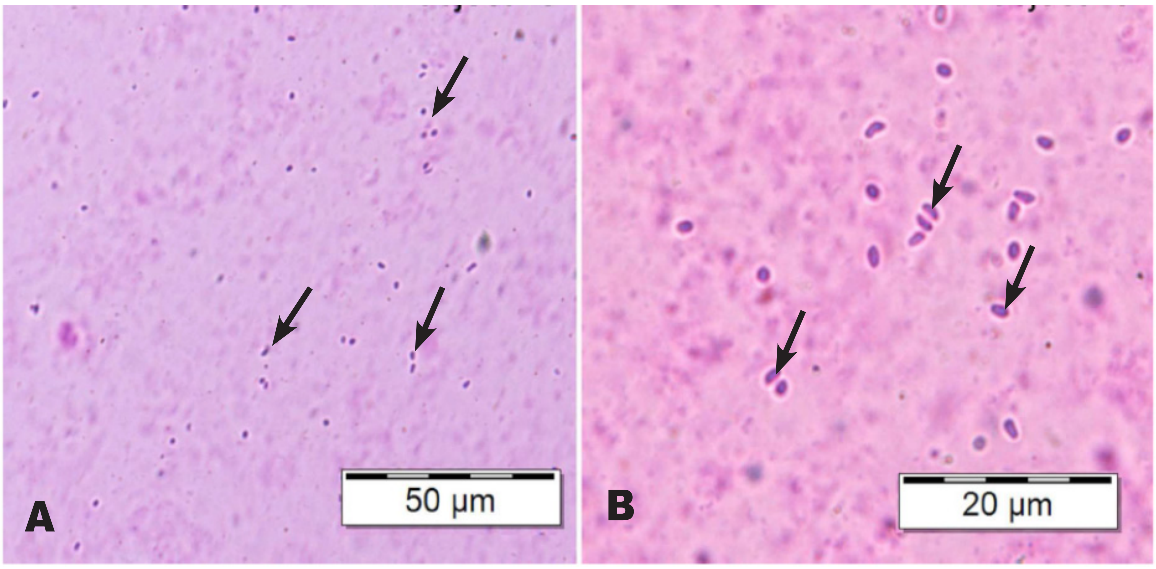

Figure 3. Spores (black arrows)of *Enterocytozoon hepatopenaei* stained with Phloxine B solution (2% w/v) examined by light microscopy at 40X (A) and 100X (B) magnification (Source: Sritunyalucksana, K. Biotec Thailand).

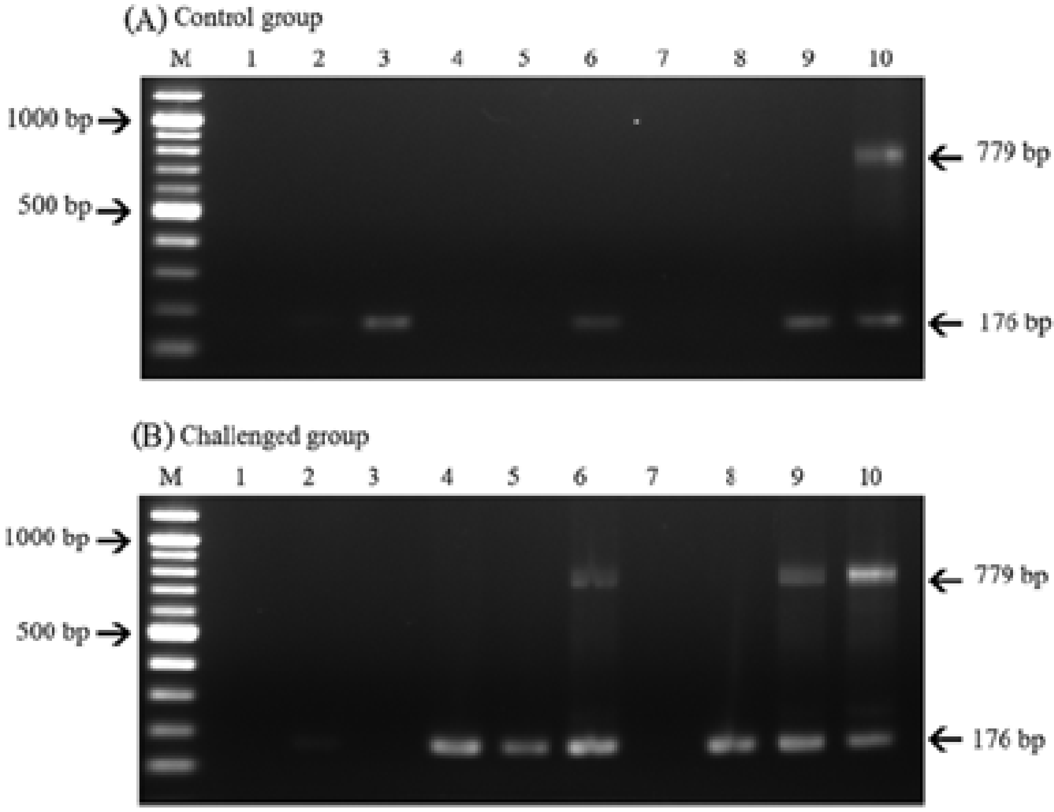

Figure 4. Agarose gels showing nested PCR microsporidian-specific amplicons using 100 ng of total DNA template from hepatopancreatic tissue obtained from *Penaeus vannamei* not challenged (A) or orally challenged (B) with the microsporidian (source: Tangprasittipap *et al.,* 2013).

Prevention and control of hepatopancreatic microsporidiosis (HPM)

At present, there is no available drug that could be practically used to treat HPM in cultured penaeids. It should be noted that EHP spores have thick walls and are not easy to inactivate. Thus, if a hatchery or grow-out farm gets infected with EHP, drastic decontamination measures should be strictly executed before restocking. In such case, disinfection of rearing water with 200 ppm chlorine has been suggested to eliminate EHP in the water. EHP-infected broodstocks could vertically transmit the spores to their offspring. Moreover, EHP-infected postlarvae could horizontally transmit the spores to the rearing water and consequently infect the yet uninfected stocks. Thus, the first and most important step that every farmer should do to avoid outbreaks of EHP infection is to ascertain that broodstock maturation facilities and hatchery facilities are clean. Because EHP has been detected in live polychaete worms and mollusks that are used as feed for broodstocks, Thai farmers have been encouraged to regularly monitor the presence of EHP in these aforementioned organisms by PCR method before use. Disinfection of eggs using chlorinated seawater has been likewise suggested. Importantly, hatchery operators have been advised to regularly clean or disinfect the rearing water and other paraphernalia such as nets, air tubes, and others, and to strictly observe biosecurity in the area.

When an outbreak of HPM occurs in a grow-out farm, farmers have been advised to contain the used water for proper disinfection prior to release. This is because effluents containing the spores released into the waterways may inadvertently contaminate the adjacent and nearby shrimp farms. Thus, to avoid and prevent the spread of EHP infection, the entire farm should be thoroughly disinfected before restocking. Liming of the pond bottom to increase the soil pH to 12 has been suggested during the pond preparation. Additionally, farmers are encouraged to maintain a bio-secure farm or closed system.

Scientific studies and programs for AHPND and HPM

In Thailand, several studies and programs have been carried out to prevent and control, and as well as mitigate the negative impacts of AHPND and HPM. These studies and/or programs include:

1. Promotion of the use of specific-pathogen-free (SPF) broodstock in order to eliminate VP_{AHPND} and EHP contamination;

2. Selective/genetic breeding program to increase *P. vannamei*'s resistance to diseases and reduction of the use of inbred lines from copy hatcheries;

3. Establishment of new shrimp farming technology to reduce the accumulation of organic wastes in the grow-out pond; and

4. Promotion of recirculating or closed systems for shrimp farming.

Way forward

The productivity of marine shrimp farming has been used as index to monitor the improvement of shrimp production in Thailand after reported production losses caused by AHPND (Figure 5). Since the first outbreak of AHPND in August 2011, the cumulative impact of AHPND on shrimp production volume over a period of 1 year, i.e. from August 2011 to July 2012 (1st phase) was not apparently pronounced based on the production data generated. On the contrary, the biggest impact was documented from August 2012 to February 2014 (second phase) as evidenced by a remarkable decrease of shrimp production volume particularly observed in February 2014. Additionally, from March 2014 to December 2015 (3rd phase) the production data showed an increasing trend, i.e. from 2.9. to 7.45 MT/1,000,000 PL, indicating that the shrimp industry of Thailand has gradually recovered from the devastating impacts of AHPND (Figure 5).

By and large, despite the negative impacts of AHPND and HPM on the shrimp industry of Thailand, all parties of the shrimp sector have been working in concert to attain the projected annual shrimp production volume of approximately 300,000 MT in 2016. Importantly, to realistically achieve the goal of maximizing the productivity and sustainability of the shrimp industry in Thailand, researchers from both the government and private sector should collaboratively act to address pressing issues related to the impact and adaptation of shrimp farming technology on climate variations,

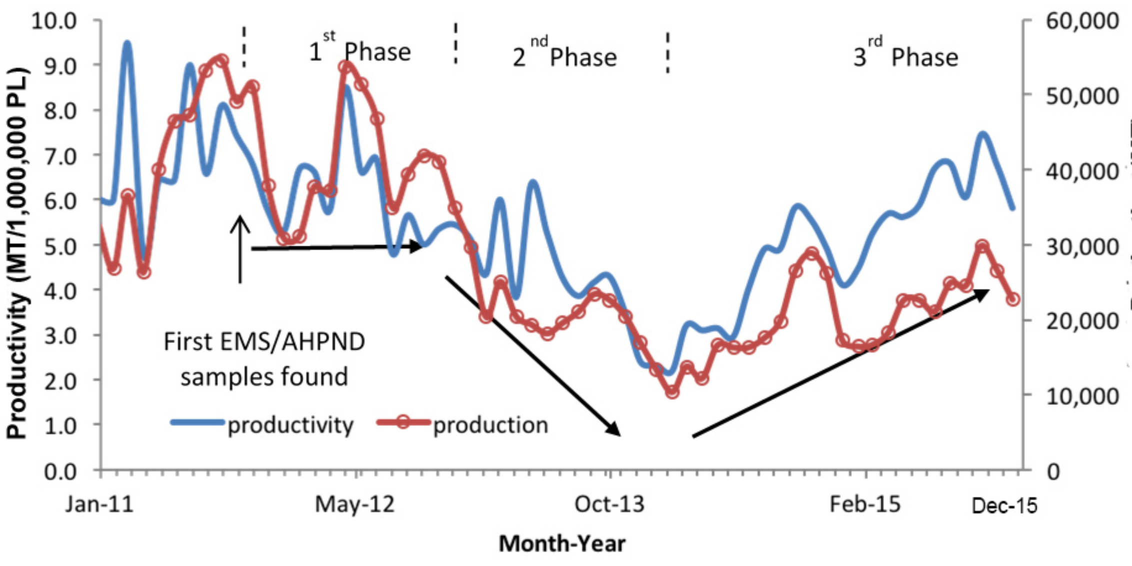

Figure 5. Productivity of shrimp farming compared with the monthly production volume during the course of acute hepatopancreatic necrosis disease (AHPND) outbreaks in Thailand from January 2011 to December 2015.

development of carrying capacity models for sustainable shrimp farming, and establishment of multitrophic aquaculture system for land-based coastal aquaculture.

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Status of Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Emerging Diseases of Penaeid Shrimps in Viet Nam

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Abstract

Acute hepatopancreatic necrosis disease (AHPND), formerly called early mortality syndrome (EMS), was first reported in 2010 among penaeid shrimps cultivated in the Mekong Delta Region of Viet Nam albeit without any laboratory confirmation. The disease subsequently spread to a wide range of shrimp production areas in the same region (Soc Trang: 1,719 ha; Bac Lieu: 346 ha; and Ca Mau: 3,493 ha), so that the Government of Viet Nam requested for technical assistance from the Food and Agriculture Organization (FAO) of the United Nations in 2011. In 2012, FAO supported Viet Nam through the project TCP/VIE/3304 *Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam*, under which the Department of Animal Health of Viet Nam (DAH) collaborated with the University of Arizona and FAO experts to carry out in-depth studies to identify the etiologic agent of the disease. As a result, unique isolates of *Vibrio parahaemolyticus* was identified as the causative agent of AHPND in 2013. Viet Nam has been vigilant and transparent with regard to aquatic animal diseases through official notifications to the World Organization for Animal Health (OIE) and the Network of Aquaculture Centres in Asia-Pacific (NACA). AHPND outbreaks have no clear temporal pattern with black tiger (*Penaeus monodon*) and whiteleg (*P. vannamei*) shrimps showing similar incidence risk. The disease occurs at any stage of shrimp cultivation, i.e. on average about 35 days after stocking. To date, unwarranted outbreaks of AHPND in major shrimp-producing provinces in Viet Nam have been apparently regulated. Aside from AHPND, white spot disease (WSD) has also been a persistent problem responsible for serious economic losses in many shrimp-producing areas in Viet Nam. To prevent and control the further spread of infectious diseases of shrimps including AHPND and WSD, multiple control measures have been implemented including guidance of farmers to improve production conditions, facilities and biosecurity application, active surveillance of shrimp production areas for early warning, screening of broodstock and postlarvae for any OIE listed diseases, regulation on movement of stocks, and collaboration with regional and international organizations in carrying out in-depth epidemiological studies that will be needed in the formulation of pragmatic and holistic disease interventions.

Introduction

Viet Nam has 28 coastal provinces with more than 3,260 km of coast line and many river systems such as the Red river and Mekong river system. These bodies of water are suitable environments for the aquaculture of various aquatic species including brackishwater shrimps. Having this advantage, the shrimp industry of Viet Nam developed rapidly and has become one of the major shrimp exporters in the world. In 2009, 2010, and 2011, the annual volume of shrimps produced in Viet Nam were 352,000 metric tons (MT), 469,893 MT, and 240,000 MT, respectively. Additionally, the shrimp products exported by Viet Nam were estimated at USD 1.5 billion, USD 1.9 billion, and USD 2.4 billion, respectively, in 2009, 2010, and 2011 (Mai, 2012).

The Mekong River Delta in Southern Viet Nam has approximately 565,000 ha of shrimp aquaculture areas contributing approximately 95% of the total volume of shrimp produced in the country. These shrimp aquaculture areas include the coastal provinces of Ben Tre, Tien

Giang, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau. The shrimp farms located in these areas have been practicing various types of shrimp culture system including intensive, semi-intensive, extensive, improved extensive, and integrated rice and shrimp culture, depending on shrimp density, type of management employed, and existing facilities and infrastructures in the farm.

While intensification of shrimp aquaculture in Viet Nam over the past several years has led to remarkable improvements in productivity, it has also been associated with disease epidemics involving bacterial, viral, fungal, and parasitic pathogens. Undoubtedly, disease has been one of the biggest constraints on production, development and expansion of Viet Nam's shrimp aquaculture industry. Among the major infectious diseases confronting Viet Nam's shrimp industry, white spot disease (WSD) and very recently, acute hepatopancreatic necrosis disease (AHPND), formerly called early mortality syndrome (EMS), have by far been implicated in serious economic losses.

The first outbreak of EMS was reported in Soc Trang province in 2010. Thereafter, EMS continuously occurred and spread to other provinces such as Tien Giang, Ben Tre, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau causing marked reduction in Viet Nam's annual shrimp production in 2011. Accordingly, researchers of the Ministry of Agriculture and Rural Development (MARD) attempted to identify the etiology of the disease and carried out concomitant practical prevention and control strategies to address the pressing problem at that time. MARD also requested assistance from international organizations such as the World Organization for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO) and Network of Aquaculture Centres in Asia-Pacific (NACA). Experts from the FAO's 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 outbreaks actually occurred since the early part of 2010 until 2011 causing significantly high mortalities among cultured black tiger and whiteleg shrimps. Based on the nature of disease spread, it was at that time speculated to be caused by an infectious agent as evidenced by the pattern of disease transmission, i.e. starting from a pond in one location and subsequently spreading to several ponds within the farm, and finally to neighboring farms.

Through the recommendation of the FAO's CMC-AH, FAO extended technical and financial assistance to implement the project TCP/VIE/3304 *Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam* with the primary aim of better understanding the etiology of the disease and identifying a number of risk management measures and key areas for future research (FAO, 2013). The DAH eventually convened and carried out in-depth investigations of the disease outbreaks. Accordingly, a total of 181 diseased shrimp samples from the three main EMS-affected provinces, i.e. Soc Trang, Bac Lieu and Ca Mau, were collected and examined. To speed up the elucidation of the etiology of the disease, the DAH also invited experts from Mahidol University, Thailand and Arizona University, United States of America, in the persons of Dr. T. Flegel and Dr. D. V. Lightner, respectively.

In mid-2011, D.V. Lightner of Arizona State University described the histopathology of both *Penaeus monodon* and *P. vannamei* obtained from many cultivation ponds in Viet Nam that were infected with EMS. The histopathology of infected shrimp was typified by massive sloughing of hepatopancreatic tubule epithelial cells that commenced in the center of the hepatopancreas and progressed outward to the embryonic (E-cell) region. Armed with this information, a more descriptive term for the syndrome, acute hepatopancreatic necrosis syndrome (AHPNS), was proposed and eventually adopted. At that time, AHPNS was still classified as an idiopathic disease because no specific causal agent had been identified yet. Fortunately, the Aquaculture Pathology Laboratory of the University of Arizona isolated the causal agent of AHPNS in pure culture in 2013 (Tran *et al.*, 2013). The causal agent of AHPNS was identified as unique strains of *Vibrio parahaemolyticus* (VP_{AHPND}) that colonized the stomach of shrimp and produced toxins that consequently caused massive sloughing of hepatopancreatic tubule epithelial cells of the hepatopancreas. When the healthy shrimps were exposed to the isolated VP_{AHPND} via immersion, 100% mortality rate was obtained coupled with the expressions of typical pathognomonic histology of AHPNS thereby fulfilling Koch's postulates (Tran *et al.*, 2013). It was also documented that cell-free broth alone obtained from the VP_{AHPND} cultures could induce the massive sloughing of the hepatopancreatic tubule cells even in the absence of bacterial cells (Tran *et al.*, 2013). Subsequent histological analyses revealed that AHPNS lesions could be experimentally induced *in vitro*, identical to those observed in AHPNS-infected shrimp samples collected from the endemic areas in Viet Nam. Following the identification of the etiologic agent, the disease was eventually named as acute hepatopancreatic necrosis disease (AHPND). Furthermore, this finding spurred the Government of Viet Nam to issue an official notification to the public regarding the etiology of AHPNS. In June 2013, FAO and DAH organized an international workshop to disseminate the outputs of the FAO-funded project.

Aside from AHPND, Viet Nam's shrimp aquaculture industry has been likewise facing serious problems associated with white spot disease (WSD) caused by white spot syndrome virus (WSSV). WSD unequivocally impacted the annual production of penaeid shrimps cultivated in major shrimp producing areas, i.e. from North to South of Viet Nam in 1994 and 1995, respectively. Because AHPND and WSD have been observed to erratically occur particularly during the early period of shrimp cultivation when shrimps are highly susceptible to these diseases, Viet Nam shrimp growers have resorted to practicing pragmatic and effective interventions to control the devastating effects of these diseases.

Status of AHPND and its economic impact

In 2010, unconfirmed outbreaks of EMS were reported in shrimp cultivation areas along Viet Nam's Mekong Delta provinces constituting the main shrimp production region. In the succeeding year, the disease consequently spread to a wide range of shrimp production areas including the provinces of Ninh Thuân (production area: 16 ha), Soc Trang (1,719 ha), Bac Lieu (346 ha) and Ca Mau (3,493 ha).

In 2012, occurrences of AHPNS did not only further spread to a wide range of shrimp production areas along the Mekong Delta provinces such as Soc Trang (production area: 2,100 ha), Tra Vinh (1,642 ha), Bac Liêu (2,000 ha), Ca Mau (4,007 ha), Ben Tre (133 ha) but also in shrimp production areas located in the middle of Viet Nam including the provinces of Quang Ngai, Binh Dinh, Ninh Thuan, Phu Yen, and Khanh Hoa.

In 2015, AHPND extensively occurred in 23 provinces of Viet Nam covering approximately 9,463 ha of the shrimp cultivation areas (1.4% of the total shrimp cultivation areas) with infected shrimps showing the typical clinical signs of AHPND. Notably, the number of affected areas abruptly increased in 2015. However, the data from 2013 to 2015 revealed that the proportion of AHPND-infected areas represented only <1.5% of the total shrimp cultivation areas (Table 1). Unwarranted outbreaks of AHPND have caused serious economic losses in Viet Nam estimated annually at less than USD 57 million. To date, though AHPND outbreaks have already been apparently regulated, minor occurrences could still be sporadically observed in some shrimp production areas in Viet Nam (Figure 1).

Shrimp species and culture stages affected

Outbreaks of AHPND have been so far documented in cultured black tiger (*P. monodon*) and whiteleg (*P. vannamei*) shrimps in Viet Nam. As shown in Table 1, the proportions of AHPND-infected cultivation areas documented from 2013 to 2015 were significantly different between black tiger and whiteleg shrimp. Whiteleg shrimp appeared to be more susceptible to AHPND than tiger shrimp based on DAH's observations over the past three years (Table 1). On average, AHPND occurred 35 days after the stocking of shrimps in cultivation ponds. However, AHPND has a wide range of occurrence period, i.e. from 0 up to 165 days and a narrower period range of 15 to 60 days post-pond stocking of shrimps (Table 2).

Table 1. Occurrence, prevalence, and impact of acute hepatopancraetic necrosis disease (AHPND) outbreaks on Viet Nam's shrimp productivity.

Year of AHPND occurrence	2013	2014	2015
No. of provinces affected	19	22	23
Total culture area (ha) affected by AHPND	5,875	5,509	9,463
P. monodon	3,300 (56.17%)	2,088 (37.89%)	5,132 (54.5%)
P. vannamei	2,575 (43.83%)	3,421 (62.11%)	4,285 (45.5%)
Prevalence of AHPND in shrimp			
P. monodon	0.53%	0.36%	0.87%
P. vannamei	5.57%	3.63%	5.06%
Total shrimp culture area (ha)	664,783	678,913	677,459
Proportion of culture area affected by AHPND	0.88%	0.81%	1.40%
Estimated losses in million USD due to AHPND	60.63	57.64	97.96

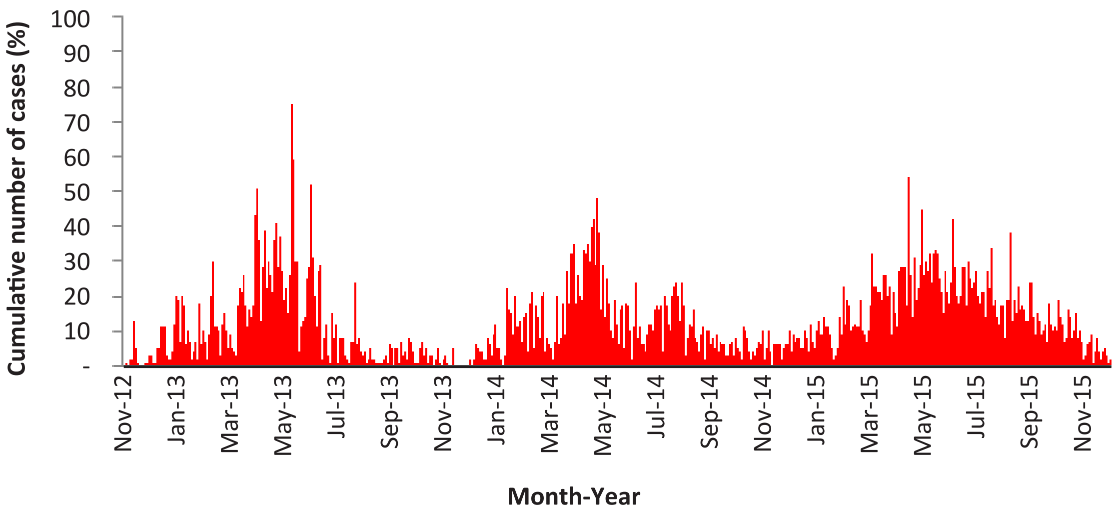

Figure 1. Temporal distribution of acute hepatopancreatic necrosis disease (AHPND) outbreaks in Viet Nam documented from 2013 to 2015.

Prevention and control of AHPND

To prevent and control the unwarranted outbreaks of AHPND, stringent biosecurity measures in both hatcheries and shrimp production farms have been strictly practiced in Viet Nam. New approaches or techniques and standards for shrimp culture such as those stipulated in the Viet Nam Good Aquaculture Practice (VietGAP) and Global Good Aquaculture Practice (GlobeGAP) have been implemented in all shrimp production areas. Active surveillance program for AHPND risk analysis has been also carried out with financial assistance extended by private farmers, local government, and the central government. For instance, the active surveillance conducted on 8 major shrimp production provinces namely Quang Ninh, Nam Dinh, Ha Tinh, Ninh Thuan, Binh Thuan, Soc Trang and Ben Tre was conducted in 2015. Active surveillance has been so far routinely carried out in all shrimp hatcheries to ensure that postlarvae are negative for shrimp pathogens before they are permitted to be transported to other shrimp cultivation areas. Every month, at least 5 shrimp postlarvae samples are obtained from small-scale hatcheries and tested for infectious diseases especially those listed in the OIE. In the case

Table 2. Incidence of acute hepatopancreatic necrosis disease (AHPND) outbreaks during the different culture stages of shrimp in grow-out ponds.

Days of culture in grow-out ponds after stocking	Year					
	2013		2014		2015	
	No. of cases of AHPND outbreaks	(%)	No. of cases of AHPND outbreaks	(%)	No. of cases of AHPND outbreaks	(%)
0-15	21	0.90	8	0.26	17	0.40
15-30	349	15.01	645	20.83	642	15.23
30-45	599	25.76	1,664	53.75	2,205	52.33
45-60	931	40.04	602	19.44	957	22.71
60-75	354	15.23	135	4.36	281	6.67
75-90	59	2.54	26	0.84	61	1.45
90-105	9	0.39	16	0.52	44	1.04
105-120	3	0.13	-	-	4	0.09
135-150	-	-	-	-	1	0.02
150-165	-	-	-	-	2	0.05
Total	**2,325**	**100**	**3,096**	**100**	**4,214**	**100**

of large-scale hatcheries, the same number of samples are taken for disease testing every week.

From January to June 2015, the DAH carried out an intensive (active) surveillance of AHPND, WSD, and hepatopancreatic microsporidiosis (HPM) in 60 hatcheries, i.e. 30 hatcheries from Ninh Thuan province plus another 30 from Binh Thuan province. In each of these randomly selected hatcheries, animal (shrimp postlarvae with mean body weight of 1.5 g) and environmental samples (water and waste) were taken and tested for the aforesaid diseases. Likewise, the DAH carried out an active surveillance of WSD, AHPND and EHP in 210 grow-out farms located in the northern (Quang Ninh, Nam Dinh), central (Ha Tinh) and southern (Ben Tre and Soc Trang) provinces. In each of these selected provinces, 30 farms were selected randomly using the designed multiple criteria approach. Similarly, specimens composed of shrimp postlarvae, water, and waste were collected and subjected to disease diagnosis. Moreover, the DAH has also implemented a passive surveillance system using a standardized reporting mechanism, i.e. from the farm level up to the national level.

One of the national strategies that has perhaps played a pivotal role in averting the further spread of AHPND among cultivated shrimps in Viet Nam was the enhancement of farmers' capacity in dealing with AHPND through a series of pertinent training courses organized by the staff of the DAH. In 2015 alone, a total of 1,500 shrimp farmers underwent the training. The training covered lectures and hands-on exercises related to shrimp health management including diagnostic methods for AHPND and corresponding good aquaculture practices, among others. To strengthen farmers' knowledge gained in the training course and ascertain that prevention and control approaches have been effectively carried out in the field, personnel of the DAH further extended field technical assistance to shrimp farmers in a collaborative manner with the local veterinary agencies.

In November 2014, the National Steering Committee for the Prevention and Control of diseases in brackishwater shrimps was established to advise the Ministry of Agriculture and Rural Development in developing and carrying out practical disease intervention and control of cultured shrimps in Viet Nam. With the protocol for prevention and control strategies in place, the program was successfully implemented in each of the shrimp-producing provinces. This was realized by enhancing the capacities of provincial veterinary personnel in conducting accurate disease diagnosis. Relative to this, the provincial veterinary laboratory facilities were accordingly improved and application of novel techniques for shrimp disease diagnosis was instituted. On June 19, 2015, Viet Nam's Law on Animal Health was approved by the National Assembly. The Law supersedes the Veterinary Ordinance 18/2004/PL-UBTVQH10, and will be effectively implemented starting July 1, 2016. The new law covers a wide range of animal health related areas including prevention, control, and surveillance of animal diseases, management of animal slaughtering and processing, import quarantine inspection of animal and animal products, and the management of veterinary drugs. By and large, shrimp farmers in Viet Nam have been encouraged to use biological and chemical products that are approved for preventing and treating diseases of cultured penaeids. Because AHPND is caused by VP_{AHPND}, farmers used antibiotics during the height of AHPND outbreaks in Viet Nam. However, the application of antibiotics in hatcheries and grow-out ponds via medicated feed did not completely prevent AHPND outbreaks. Additionally, in compliance with the regulations on antibiotic residue and food safety, shrimp farmers were instructed to strictly follow the correct dosage of antibiotic administration and concomitant withdrawal period.

Status of white spot disease (WSD) in cultured shrimps

In 2015, WSD occurred in 23 provinces covering a total area of approximately 5,396 ha (0.79% of the total of cultivation area) in Viet Nam. The number of infected area decreased sharply compared to the data generated in 2013 and 2014, i.e. the proportion of infected to the total cultivation area was less than 4% (Table 3). While WSD occurred annually from January to September in 2013, 2014 and 2015, serious outbreaks however so far recorded usually commenced in March and seemingly peaked in May (Figure 2).

Table 3 clearly shows that the proportion of WSD-infected cultivation areas were significantly different between the black tiger and whiteleg shrimps. Whiteleg shrimp was seemingly more susceptible to WSD than black tiger shrimp (>3.9 times) throughout the course of our 3-year investigation. This could be attributed to the fact that the black tiger shrimp is a local species, hence they could easily adapt to the local environment whereas the whiteleg shrimp was imported from other countries. On average, shrimps could be infected by WSD at 35 days after stocking in grow-out ponds. It should be noted however, that WSD has a wide range of occurrence period, i.e. from 0 up to

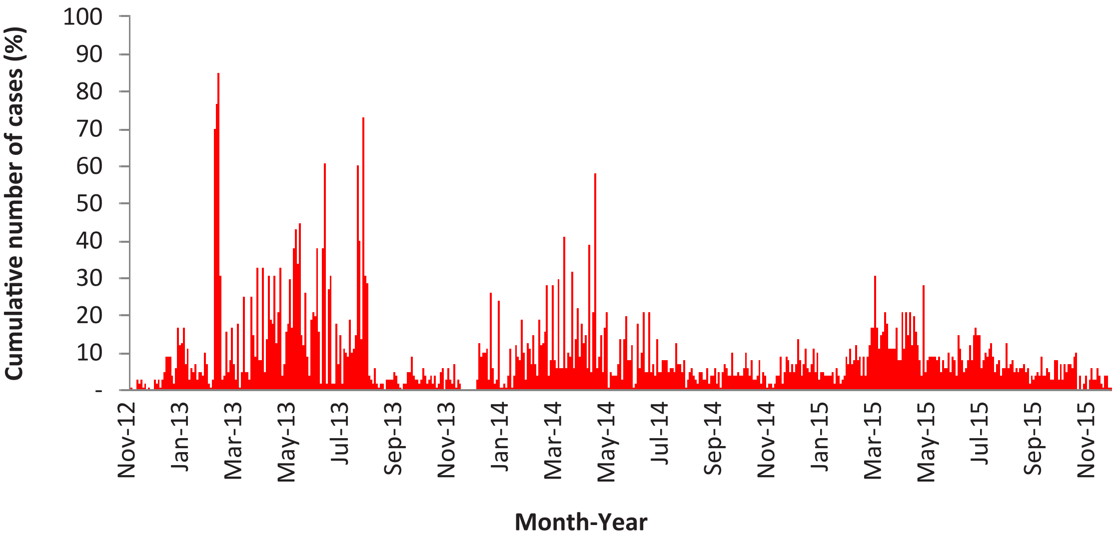

Figure 2. Temporal distribution of white spot disease (WSD) outbreaks in Viet Nam documented from 2013 to 2015.

Table 3. Impact of white spot disease (WSD) outbreaks on Viet Nam's shrimp productivity.

Year of WSD occurrence	2013	2014	2015
No. of provinces affected	28	23	23
Total culture area (ha) affected by WSD	12,352	23,850	5,369
P. monodon	7,677 (62.16%)	14,660 (61.47%)	3,447 (64.19%)
P. vannamei	4,674 (37.84%)	9,190 (38.53%)	1,923 (35.81%)
Prevalence of WSD in shrimp			
P. monodon	1.24%	2.51%	0.58%
P. vannamei	10.12%	9.76%	2.27%
Total shrimp culture area (ha)	664,783	678,913	677,459
Proportion of culture area affected by WSD	1.85%	3.51%	0.79%
Estimated losses in million USD due to WSD	127.47	249.54	55.58

Table 4. Incidence of white spot disease (WSD) outbreaks during the different culture stages of shrimp in grow-out ponds.

Days of culture in grow-out ponds after stocking	Year					
	2013		2014		2015	
	No. of cases of WSD outbreaks	(%)	No. of cases of WSD outbreaks	(%)	No. of cases of WSD outbreaks	(%)
0-15	18	1.03	5	0.31	7	0.33
15-30	294	16.83	345	21.63	362	17.05
30-45	499	28.56	854	53.54	1019	48.00
45-60	635	36.35	267	16.74	543	25.58
60-75	280	16.03	84	5.27	121	5.70
75-90	15	0.86	20	1.25	42	1.98
90-105	3	0.17	17	1.07	17	0.80
105-120	1	0.06	3	0.19	5	0.24
135-150	2	0.11	-	-	5	0.24
150-165	-	-	-	-	1	0.05
210-225	-	-	-	-	1	0.05
Total	**1,747**	**100**	**1,595**	**100**	**2,123**	**100**

225 days after stocking. Moreover, Vietnamese farmers have otherwise experienced a narrower period of WSD occurrence, i.e. ranging from 15 to 60 days after stocking (Table 4).

WSD-infected shrimp exhibit lethargy, empty or little food in gut, swimming on the surface water layer, pale shell color and typical white spots (0.2-0.5 cm) on the head and the tail cover. Viral genome-based detection methods including PCR and loop-mediated isothermal amplification (LAMP) have been employed as confirmatory tests for the detection of WSD in asymptomatic and diseased shrimps. To prevent and control WSD outbreaks among cultured penaeids, similar strategies used for AHPND have been likewise practiced in Viet Nam.

Way forward

Occurrence of AHPND and WSD has by far been documented in almost all stages of shrimp cultivation with higher frequency of occurrence being observed among the months of March to July in Viet Nam. Whiteleg and black tiger shrimps could be both infected with AHPND and WSD with the former species appearing to be seemingly more susceptible to these diseases. To date, hepatopancreatic microsporidiosis

(HPM) caused by *Enterocytozoon hepatopenaei* (EHP) has not been detected in cultured penaeids in Viet Nam based on the results of the active surveillance conducted in 2015 and 2016, respectively. Viet Nam will continue its vigilance and transparency with regard to aquatic animal diseases through official notifications to the OIE and NACA. Also, the DAH of Viet Nam will strictly continue implementing its comprehensive policy system on disease prevention and control in cultured shrimps. Thus, to efficiently prevent and control the entry and further spread of newly emerging and persistent infectious diseases of cultured penaeids including AHPND and WSD, respectively, multiple control measures will be continually implemented including the guidance of farmers to improve production conditions, facilities and biosecurity application, active surveillance of shrimp production areas for early warning, screening of broodstock and postlarvae for any OIE listed diseases, regulation on movement of stocks, and collaboration with regional and international organizations in carrying out in-depth epidemiological studies that will be needed in the formulation of pragmatic and holistic disease interventions.

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ANNEX 2

Summary of Workshop Discussions[1]

The Technical Workshop was undertaken based on the country and expert presentations on the status, recent issues and gaps on the management, prevention and control of AHPND (and other shrimp diseases), as well as from the discussions and comments made by the participants. The aim of the Technical Workshop was to formulate interventions and recommendations to address the important issues identified in the ASEAN Member States (AMS). The workshop mainly focused on early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) with few comments and suggestions for hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (HPM-EHP).

Based on the inputs from AMS representatives, private sectors and invited experts, the following issues were considered for future actions and studies on AHPND and HPM-EHP:

Acute hepatopancreatic necrosis disease (AHPND)

- Use of live feeds for broodstock (specifically polychaetes), as these were proven to be carriers of the pathogen;
- Copy specific-pathogen-free (SPF) shrimp (*Penaeus vannamei*) hatchery - effects on inbreeding or genetic erosion on susceptibility to AHPND; breeding programs are long-term, usually highly expensive and need sufficient resources; therefore, these programs should be carefully planned, efficiently implemented and sustained;
- Misconception about SPF shrimps and its use; ban on SPF broodstock and live shrimp products from AHPND-affected countries;
- Vertical transmission of AHPND bacteria (broodstock to postlarvae);
- Toxin plasmid transfer to other *Vibrio* species and possibly other bacterial pathogens that are common in the aquatic/rearing environment; *V. harveyi* and *V. owensii* were already reported to carry the AHPND toxin plasmid;
- Environmental risk factors for spread and outbreak of AHPND;
- Mixed infection with other shrimp pathogens - covert mortality nodavirus (CMNV), EHP, white spot syndrome virus (WSSV);
- Efficacy of green water technology in prevention of AHPND; this is currently being practiced in Viet Nam and the Philippines with some degree of success;
- Issue on extensive/non-registered farms: risks that these farms may pose to the spread and occurrence of the disease;
- Probiotics: locally produced vs. imported; issue on banning probiotics for use in prevention of AHPND;
- Biosecurity capacities of countries to prevent the entry of the pathogen;
- Emergency preparedness and contingency planning;
- Sharing of information and experiences among countries affected and not affected by the disease;
- Lack of disease surveillance in processing plants and the wild population
- Antimicrobial resistance;
- Certification of Aquatic Animal Health (AAH) professionals (other than veterinarians); and,
- Cooperation of government and producers in prevention and management of AHPND; strengthen government and private sector partnership; learn from the farmer experience and understand the science behind and disseminate.

[1]Prepared by Dr. Eduardo Leaño, NACA

Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (HPM-EHP)

There was not much discussion on how to manage this disease, except on one comment from Indonesia about the detailed procedure for the Wet Mount Method developed by Prof. Timothy Flegel's team (Mahidol University, Thailand). Some of the recommendations given on AHPND can also be applied for this emerging disease, particularly on border control, emergency preparedness and contingency planning. Specific researchable areas at present, as recommended by Prof. Flegel, to better understand the pathogen involved and the disease mechanism include the following:

- Identification of the reservoir of the pathogen to include live feeds and other crustaceans that are common in the aquatic environment;
- Detailed study on the effect of the pathogen on growth and survival of infected shrimps;
- Preventive and control measures; and
- Coinfection with other important pathogens of shrimps (viral and bacterial), and its association to other shrimps diseases (e.g. white feces syndrome, AHPND).

The suggested interventions and recommendations to address the above issues are summarized in the Matrix Table below.

Summary of Interventions and Recommendations to Address the Issues

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
Live Feeds (specifically polychaetes)	Use of live feeds for broodstock (specifically polychaetes)-as these were proven to be carriers of the pathogen	Survey of polychaetes (*Marphysa* sp.) in the pond environment – Assess susceptibility to AHPND and other pathogens	Study on transmission of AHPND bacteria from polychaetes to shrimp (to be completed before any intervention should be done)	SEAFDEC/AQD, NACA and concerned AMS	S to M
		Preference for polychaete species living in mud sediment (than those in sandy sediment)	Refine sampling procedures for PCR detection of AHPND bacteria		
		Use of non-live polychaete (frozen, pasteurized, etc.)	Develop genetic markers on susceptibility of polychaetes to AHPND bacteria		

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
		Trace source of polychaetes; processing techniques for live polychaetes to reduce bacteria load prior to feeding: -depuration, disinfection and other treatments	Research on mass production of polychaetes in a more biosecure environment		
		Develop regulations on importation of polychaetes	Develop and implement regulations on importation of live polychaetes to include health certificate (absence of major shrimp pathogens)		
SPF Broodstock	Copy SPF (*P. vannamei*) hatchery - effect on inbreeding/genetic deterioration on susceptibility to AHPND	Assess possible inbreeding/genetic deterioration in *P. vannamei* and correlate with disease susceptibility and outbreaks	Assess existing stocks (especially broodstock) of *P. vannamei* for signs of inbreeding/genetic deterioration	AMS, SEAFDEC/ AQD, Donor agencies, and NACA	M to L
	Breeding programs are usually long-term, highly expensive and need sufficient resources, therefore, they should be carefully planned and efficiently implemented and sustained		Need to develop molecular genetic markers for disease resistance: Marker-assisted breeding		
	Misconception about SPF shrimps and its use		More understanding on SPF/SPR/SPT to prevent bringing in exotic diseases – provide guidance to government	AMS, SEAFDEC/ AQD, NACA	S

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
	Ban on SPF broodstock and live shrimp products from AHPND-affected countries	Information awareness on what SPF really is in terms of its health status (under biosecure facilities and under farm-level environments); to include information on "High-Health" shrimps	Disseminate relevant information on SPF, SPR and SPT		
Pathogens and Disease Outbreak	Vertical transmission of AHPND bacteria (broodstock to post larvae)	Clear understanding on VP_{AHPND} vs other strains of Vp.	Study on vertical and horizontal transmission of AHPND bacteria	Concerned AMS, SEAFDEC/ AQD	M
	Toxin plasmid transfer to other *Vibrio* species and possibly other bacterial pathogens that are common in the aquatic/rearing environment	Detect AHPND-associated toxin plasmid in other *Vibrio* species and other bacterial pathogens	Study on the ecology of vibrios under the *Vibrio harveyi* clade where *V. parahaemolyticus*, *V. harveyi* and *V. owensii* (all found to harbor the toxin plasmid) belong	SEAFDEC/ AQD, AMS Research Institutes	M
	V. harveyi and *V. owensii* were already reported to contain the AHPND toxin plasmid		Study on conditions that will trigger the virulence of AHPND bacteria		
	Environmental risk factors for spread and outbreak of AHPND		Study on environmental conditions that facilitate plasmid transfer, and on virulence of plasmid-containing bacteria to susceptible shrimps species		

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
	Mixed infection with other shrimp pathogens (CMD, EHP, WSSV)	Interventions on water quality management to maintain low levels of AHPND bacteria	Implement BMPs focusing on water and feeding management	AMS, Private Sectors	S
		Screen AHPND-affected shrimp samples for presence of other pathogens to check mixed infection	Multi-infection study: effect on mortality and histopathological features of the hepatopancreas	SEAFDEC/ AQD, AMS Research Institutes	S to M
Grow-out; Preventive Measures	Efficacy of green water technology in prevention of AHPND (being practiced in Viet Nam, Philippines)	Review previous results of the technology on prevention of luminous vibriosis in *P. monodon*; many detailed studies were already undertaken, especially on its effect on microbiota in the pond environment	More science-based analysis on the mechanisms involved in green water technology which might be directly correlated to prevention of AHPND	Concerned AMS	S
	Issue on extensive/ non-registered farms: risk that they may pose to the spread and occurrence of the disease	More attention and guidance should be provided to small scale/extensive culture farmers/ sectors as they represent the weak link in the system posing high risk for diseases. These include (but not limited to) non-registered farms, farmers from rural communities, and small-scale farms (in terms or farm area) practicing monoculture of shrimps	Promotion of public awareness programs to encourage small scale farmers to share/report disease outbreaks (if any) to nearest authority in their area	Concerned AMS	S

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
	Probiotics? Locally produced vs. imported probiotics; Probiotic ban	Understanding how probiotics work in the aquatic environment and its relation in preventing AHPND occurrence	Proper research needed on the efficacy and utilization of locally-produced probiotics	Concerned AMS, SEAFDEC AQD	S to M
		Use of probiotics with proven efficacy in reducing the risk for AHPND occurrence	Researchable areas on its application on different targets such as pond bottom, water, shrimp and feed incorporation		
Border Control and Biosecurity	Biosecurity capacities of countries to prevent the entry of the pathogen	Establishment or improvement of quarantine systems, especially at the border control facilities	ASEAN: Develop a legal framework for the implementation of the ASEAN Guidelines on SOP for Responsible Movement of Live Aquatic Animals	SEAFDEC, NACA and AMS	M to L
		Revisit or review existing national policies and regulations for inclusion of aquatic animal health	Implementation of ASEAN Shrimp GAP and GAqP by AMS, in line with their respective national GAPs (if any)		
	Emergency preparedness and contingency planning	Develop and/or implement national aquatic animal health management strategies for appropriate emergency response during any disease outbreaks (including known and emerging diseases)	Capacity building on emergency preparedness and contingency planning, especially among less developed countries in the ASEAN	AMS, FAO, SEAFDEC	M to L

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
Other Issues	Sharing of information and experiences among countries affected and not affected by the disease	Encourage farmers (especially private sectors, with successful experiences in preventing AHPND) to share their experiences to relevant authorities and other shrimp farmers including effective preventive measures, important interventions to control spread of the disease	Information sharing and education programs for shrimp farmers	SEAFDEC/ AQD, NACA, Private Sectors, AMS	M to L
	Lack of disease surveillance in processing plants and the wild population	Countries should consider to include processing plants (especially processing wastes) in AHPND surveillance	Develop national regulation on surveillance of processing plants (for shrimps and other aquatic products) Follow and implement OIE guidelines on disease surveillance among wild populations	Concerned AMS, FAO, SEAFDEC/AQD, NACA	S to M
	Antimicrobial Resistance	Implementation of appropriate regulations on the use of veterinary drugs and other chemicals	Assist in the implementation of and strict compliance to ASEAN Guidelines on the Use of Chemicals in Aquaculture Assessment of antimicrobial resistance of major bacterial pathogens of shrimps (and fish)	AMS	M to L

Acute hepatopancreatic necrosis disease (AHPND)					
Stage/Phase	**Problems (Issues)**	**Possible intervention (priority areas from 2016 onwards)**	**Recommendations**	**Responsible Agency**	**Timeline S (Short term): 1-2 years M (Medium): 2-5 years L (Long): > 5 years**
	Certification of Aquatic Animal Health (AAH) Professionals (other than veterinarians)	Need to develop and implement Guidelines/ Certification Scheme for registration and accreditation of AAH professionals	Assist national governments in developing Guidelines/ Certification Scheme for registration and accreditation of AAH professionals	AMS, NACA, OIE, FAO	M to L
	Cooperation of government and producers in prevention and management of AHPND. Strengthen government and private sector partnership. Learn from the farmer experience and understand science behind and disseminate	Strengthen public and private partnership Efficient communication between researchers and government (Competent Authority) on research updates/ findings for proper dissemination to private sectors (if necessary)	Prepare checklist/ guidelines for the farmers such as risk factors and risk management practices coming from AHPND affected countries	AMS, Private Sectors, Key Research Institutes, SEAFDEC/AQD	S to L

About SEAFDEC

The **Southeast Asian Fisheries Development Center (SEAFDEC)** is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member countries are Brunei Darussalam, Cambodia, Indonesia, Japan, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Viet Nam.

The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of the member countries.

SEAFDEC has five departments that focus on different aspects of fisheries development:

- The **Training Department** (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- The **Marine Fisheries Research Department** (MFRD) in Singapore (1967) for post-harvest technologies
- The **Aquaculture Department** (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- The **Marine Fishery Resources Development and Management Department** (MFRDMD) in Kuala Terrengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries, and
- The **Inland Fishery Resources Development and Management Department** (IFRDMD) in Palembang, Indonesia (2014) for sustainable development and management of inland capture fisheries in the Southeast Asian region.

AQD is mandated to:

- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information

AQD maintains four stations: the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo Province; the Igang Marine Station in Guimaras province; and the Binangonan Freshwater Station in Rizal province. AQD also has a Manila Office in Quezon City.




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