Advocating Preventive Measures that Inhibit Early Mortality Syndrome in Shrimps

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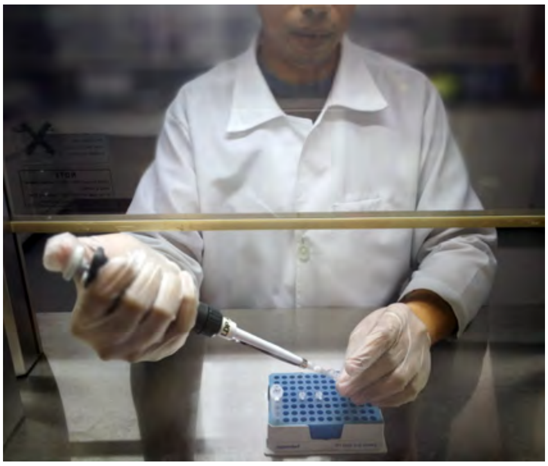
Early Mortality Syndrome (EMS) is a generic name used to describe the observed mortality occurring within the first 30 days of stocking shrimp post larvae (PL) in ponds. The aquaculture species reported to be affected by EMS are *Penaeus monodon*, *P. vannamei* and *P. chinensis*, of which *P. monodon* is the most susceptible. EMS has been reported in China (2009), Viet Nam (2010), Malaysia and Borneo (2011), and Thailand (2012). EMS could have been present in the Philippines as early as 2007 but this was not given attention then. Fish farmers in the Philippines observed that mortalities that occur as early as one week after stocking *P. monodon* PL in ponds or within two months of stocking were not due to the whitespot syndrome virus (WSSV). Hence, the farmers call it the two-month mortality syndrome (Tendencia *et al*., 2014). EMS is associated with WSSV, microsporidian infestation, *Vibrio* infection, and chemical contamination (Flegel, 2016; FAO, 2013). Affected shrimps have pale to whitish hepatopancreas with black spots or streaks, rigid and hard to squash. Histopathology of the hepatopancreas of shrimp samples from EMS cases showed massive necrosis and sloughing or shedding of the epithelial cells. EMS characterised by these specific histopathological changes in the hepatopancreas is called Acute Hepatopancreatic Necrosis Disease (AHPND). Incidence of AHPND has been recently reported in China, India, Malaysia, Mexico, Philippines, Thailand, and Viet Nam.

EMS/AHPND is due to a bacterium, *Vibrio parahaemolyticus* (VPAHPND) that colonizes the shrimp stomach where it produces the toxin responsible for the histopathological changes in the hepatopancreas. *V. parahaemolyticus* is ubiquitous and ever-present in marine and brackishwater environments, and thus can be present in the cultured shrimp,

Healthy *P. monodon* harvested from a local fishpond in the Philippines

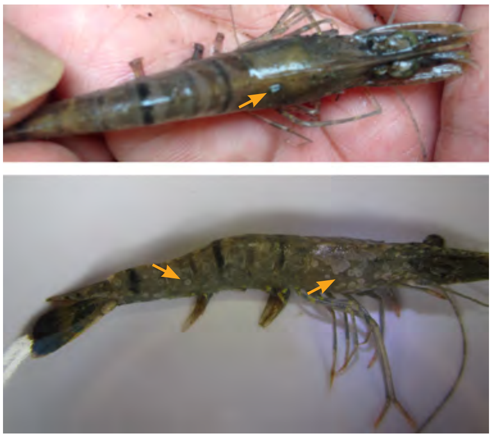


Fish health officer preparing samples for polymerase chain reaction (PCR) analysis

water, sediments, and associated organisms of the culture pond. VPAHPND have transferrable plasmid or jumping genes that carry the PirA/B toxins (Kondo *et al.*, 2015). In this case, the jumping genes that carry the toxin, leap into other bacteria in the environment resulting in the variation of the *V. parahaemolyticus* that has been isolated from shrimps experiencing an AHPND outbreak, as well as in the isolation of *V. harveyi*-like, *V. Owensii*, and *V. campbelii* in EMS/AHPND cases (Kondo *et al.*, 2015; Liu *et al.*, 2015; Dong *et al.*, 2017).

Incidence of AHPND and WSSV in Shrimp Aquaculture

Between 2013 and 2014, production volumes of *P. vannamei* and *P. monodon* in the Philippines decreased due to disease outbreaks primarily in Luzon and the Visayas areas (Bureau of Agricultural Statistics, 2014), which was suspected to have been caused by AHPND. Samples from shrimp farms were examined for the toxin-producing strain of *V. parahaemolyticus* because of the AHPND-like symptoms occurring in shrimps after 56-94 days of culture. Analysis using the IQ2000 AHPND/EMS Toxin 1 PCR test generated 218 bp and 432 bp amplicons, confirmative of the toxin-producing strain of *V. parahaemolyticus* among shrimps taken from eight of the nine ponds sampled. In *P. monodon*, histology revealed a massive sloughing of undifferentiated cells of the hepatopancreatic tubule epithelium in the absence of basophilic bacterial cells. The tests using the Polymerase Chain Reaction (PCR) generated the two amplicons confirmatory for AHPND among shrimps sampled from five of seven ponds, confirming the



(Top): *P. monodon* with AHPND. Note the whitish color of the hepatopancreas; (bottom): *P. monodon* infected with WSSV. Note the white spots on the abdominal segments and carapace

presence of AHPND in *P. vannamei* and *P. monodon*, and suggesting that the disease can also impact late-stage juvenile shrimps (dela Peña *et al*., 2015).

Starting in 2013, AHPND was already included in the NACA/FAO Quarterly Aquatic Animal Disease (QAAD) Reporting System under the "non-OIE listed diseases" category for crustaceans. A year later, a request was submitted to the World Organization for Animal Health (OIE) to include AHPND in the List of Notifiable Diseases. However, the OIE Aquatic Animal Health Standards Commission (AAHSC) did not endorse the listing, and AHPND was not included in the OIE List until January 2016.

On the other hand, WSSV has already been included in the OIE List since 1997, and as of 2018, both AHPND and WSSV have been included in the OIE listed diseases for crustaceans. It should be noted that diseases that have not been reported for two consecutive years are delisted from the OIE List.

Cross sectional studies have been carried out by several researchers, where the results identified the factors that may increase or reduce the risk of AHPND and WSSV. These are related to the pond characteristics, season, pond preparation protocol, water management, culture practices, farm inputs, and water parameters (Bondad-Reantaso, 2016; FAO, 2013; Tendencia *et al*., 2011; Tendencia *et al*., 2010a; Tendencia *et al*., 2010b). Listed in **Table 1** are the factors that increase the risks of AHPND and WSSV in *Penaeus monodon*, while the factors reducing the risks are listed in **Table 2**.

Preventive Measures for Mitigating the Incidence of AHPND and WSSV in Shrimp Aquaculture

The Japanese Trust Fund (JTF) has been providing financial assistance to AQD to carry out research studies towards the development and/or improvement of preventive measures against AHPND and WSSV. The studies include identification of disease risks and protective factors, while tank-based and pond studies were also carried out to mitigate the effects of AHPND and WSSV in cultured *P. monodon*.

These JTF-funded studies, include: 1) Epidemiology of the White Spot Syndrome Virus (WSSV) in Different Shrimp (*P. monodon*) Culture Techniques in the Philippines; 2) Establishment of Management Technology for Disease Tolerant and Sustainable Aquaculture Environment; 3) Epidemiology of the Early Mortality Syndrome in *P. monodon*; and 4) Responsible Aquaculture through Aquasilviculture.

Table 1. Factors that increase the risks of AHPND and WSSV in *Penaeus monodon*

	AHPND	WSSV
Pond description	• Bigger pond size • Near AHPND-affected ponds	• Near other pond
Season	• Warm season	• Cold season
Pond preparation	• Sludge removal • Pond drying • Use of saponin	• Sludge removal
Water management	• Low water turnover	• Sharing of water source with other pond • Same receiving and water source • Tidal
Culture method	• Source of PL • High stocking density • Intensive system • Semi-closed system	• Source of PL • High stocking density
Feed and other inputs	• Use of antibiotics, vitamins, minerals • Live feeds • Overfeeding • Fertilizer, molasses use resulting in high nutrient levels	• Feeding with live mollusks
Water parameters	• Temperature fluctuations • pH 8.5- 8.8 • Low salinity	• Temperature fluctuation • pH fluctuation • Low salinity

Table 2. Factors that could reduce the risks of AHPND and WSSV in *Penaeus monodon*

	AHPND	WSSV
Pond description	• Older pond • With reservoir	
Months/Season	• Cold	• Warm
Pond preparation	• Chlorination and liming	
Water management	• Use of aged seawater, held > 35 days before use	
Culture method	• Greenwater, • Biofloc • Use of probiotics	• Greenwater • Presence of mangrove
Feed and other inputs	• Reduced/controlled feeding	• Abundance of natural food
Water parameters	• Low salinity	

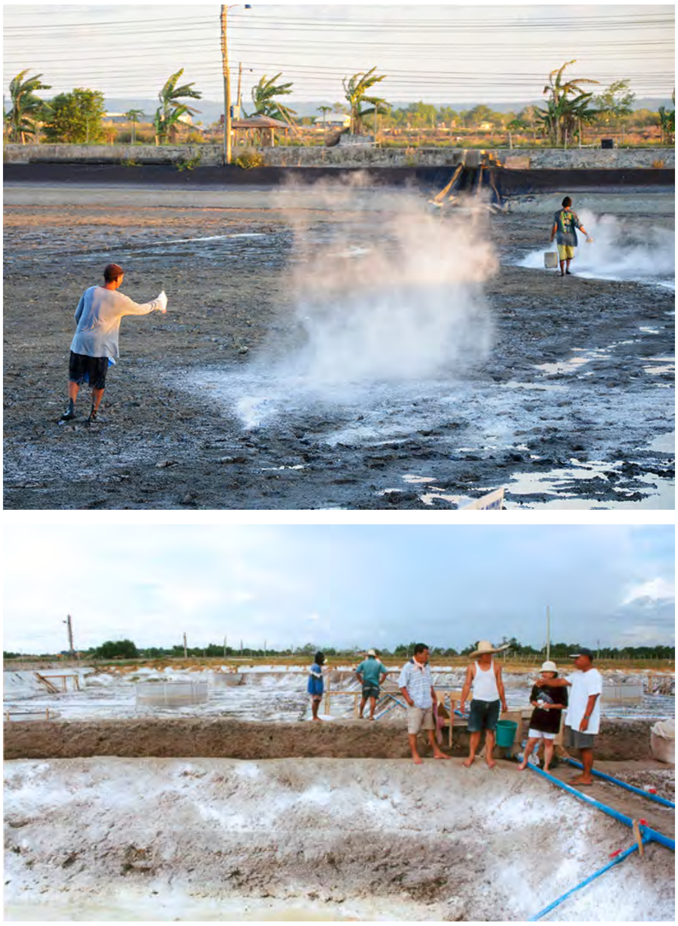
Aside from the epidemiological and disease prevention studies, the JTF also funded studies on shrimp diseases surveillance and detection methods, and in the recently-completed study "Monitoring and Surveillance of Transboundary Pathogens in Cultured Shrimps and Freshwater Prawn," *P. monodon* and *P. vannamei* were tested for viral diseases using PCR on WSSV, the Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Taura syndrome virus (TSV), and the Infectious Myonecrosis Virus (IMNV). The results showed that only IHHNV and WSSV affect shrimps cultured in the Philippines, while the other two viral diseases, *i.e.* TSV and IMNV are presumed exotic to the country. The ongoing study "Development and Acceleration of Rapid and Effective Fish and Shrimp Health Management" attempts to determine the (viral DNA/RNA copies in an organism that can result in an infection) threshold infection levels for WSSV and other pathogens such as VPAHPND using q-PCR.

Despite the identification of AHPND and WSSV risk and protective factors, EMS continues to bring havoc to the shrimp industry as evidenced by their inclusion in the OIE List of Notifiable Diseases in 2018. This calls for the development and/or improvement as well as intensified promotion of preventive measures against EMS, which include strict and proper implementation of existing biosecurity and good aquaculture practices (GAPs). Ecosystem approach to aquaculture to prevent diseases should be practiced by small-scale shrimp farmers, whose ponds are located very close to each other, sharing the same water source, and lacking funds to rehabilitate their ponds or to purchase and use probiotics.

Dead shrimp in ponds: mortality is due to WSSV

Promotion of Good Aquaculture Practices

Implementation of GAPs begins with site selection. It is generally perceived that most ponds had already been in operation, therefore, the identified risk factors related to pond site selection, *i.e.* near to other ponds and big pond size could not be avoided. Proper pond preparation is the next step to avoid EMS occurrence (Tendencia and Coniza, 2015). One of the identified risk factors to both AHPND and WSSV is sludge removal. Proper sludge removal is one of the aspects in GAPs. However, this is not properly followed by most shrimp farmers when depositing the sludge on the pond dike. Sludge removed from the pond bottom usually contains toxic substances, high organic load, and microorganisms that could be washed back into the culture pond. Thus, sludge removed from pond bottom should be placed far from the pond site. After sludge removal, the pond bottom should be ploughed and cracked dry to oxidize the remaining organic matter and other toxic substances, and then limed to kill harmful microorganisms. Liming increases the pH of wet soil to 11, to efficiently kill harmful microorganisms including viruses, and shift the dominance of vibrios from green to yellow colonies. The yellow vibrios usually have probiotic effect while the green ones are pathogenic. Furthermore, lime application to soil with pH 11 can kill unwanted species (Coniza and Tendencia, 2014). Most small-scale shrimp farmers with bigger ponds could not totally dry their ponds due to uneven pond bottom and do not apply lime due to budgetary constraints. Moreover, good plankton growth should be fostered through the application of fertilizers. Plankton not only improves water quality but also enhances the shrimp immune system making them more resistant to EMS.



Application of lime in shrimp ponds

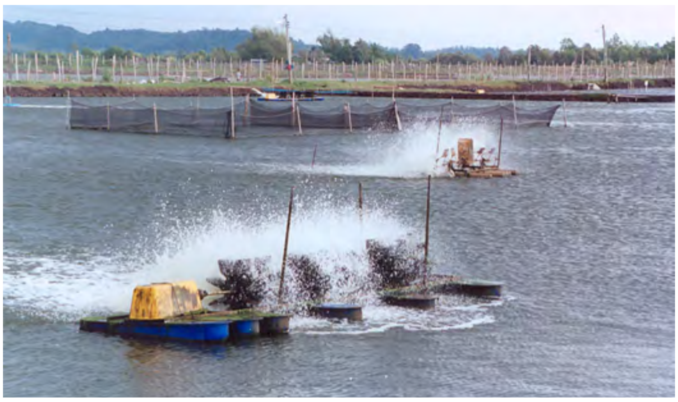
Good water management is another aspect of the GAPs. The water used to fill the culture pond should be filtered to prevent entry of unwanted species that could be disease carriers. The risk brought by sharing water source with other ponds, and having the same water receiving and source could be mitigated by providing reservoir and settling ponds (Tendencia *et al*., 2011). The reservoir can be stocked with high saline tilapia or other finfishes such as siganids or rabhbitfish. After >14 days, the water from the reservoir with finfish which is known as greenwater system, can be used to culture shrimp (Tendencia, 2018a; Tendencia *et al*., 2015). The fish faeces in a greenwater system would also serve as fertilizer to promote the growth of algae, as previously mentioned, to help improve the water quality and shrimps' resistance to diseases like EMS.

Furthermore, algae and fish mucus also have antimicrobial properties. If the reservoir is not stocked with finfish, the water could be stocked in the reservoir for at least 28 days to make it microbiologically mature before using it to fill the shrimp culture pond. Nevertheless, the effluents from shrimp ponds should be emptied into a settling pond with mollusks, macroalgae or mangroves before draining the water into the sea after > 14 days (Tendencia, 2018b; Tendencia *et al*., 2012). Mollusks, macroalgae, and mangroves have the ability to remove nutrients from the effluents aside from having anti-microbial properties.

Adoption of semi-intensive/intensive culture system

The pond is ready for stocking when there is good growth of natural food. Shrimp PL for stocking should be uniform in size and previously analysed to be AHPND and WSSV free. High stocking densities should be avoided as these would easily pollute the pond and stress the shrimps making them susceptible to diseases like EMS. In addition to causing stress, high stocking density increases the frequency of contact among individual stock, leading to increased rates of disease transmission and infection. On-farm nursery rearing of shrimp PL inside hapa nets for 15-20 days will help the shrimps adapt to the new environment where they are exposed to a lot of micro-organisms and a range of environmental parameters as opposed to the controlled condition in hatchery tanks (Rodriguez *et al*., 1993).

During culture, water depth should be maintained to at least 80 cm in the shallowest part of the pond, but > 100 cm is better. Transparent water should be avoided, as transparency is a gauge to determine abundance of not only natural food but also of suspended solids. Water exchange should be minimized and should be done during critical period only to avoid the EMS risk factors from occurring, such as fluctuations in pH, temperature, and salinity. Agricultural lime can be applied after water exchange and rain, and paddle wheels should be used to avoid low dissolved oxygen (DO) level. Feeding trays should be installed to monitor feed consumption, while feed should be based on the demand of the shrimp to avoid uneaten feeds that can pollute the environment and increase the organic matter contents of the pond soil, because pathogens causing EMS thrive well in an environment with high organic load. Aside from the high organic load in the soil, uneaten feed can lead to increased nitrogen in the pond water to levels detrimental to the shrimp. If live food such as trash fish and mollusk are used as feed to the shrimp, these should be pathogen free.



Use of paddle wheel aerators in shrimp pond

Optimizing the use of biosecurity measures

Although biosecurity is one of the protective measures that farmers could rely on to prevent disease occurrence by excluding pathogens and carrier organisms from the culture environment, this did not prevent WSSV outbreak in a cross sectional study by Tendencia *et al*. (2011). This may be because in many cases biosecurity measures, do not achieve their purpose. Nevertheless, optimization of the biosecurity measures in ponds is still necessary. An effective biosecurity program requires careful planning and strict implementation of the measures. Pathogen exclusion in shrimp farm needs technical knowledge and experience in order to identify the various pathways, as pathogen would take all possible ways to infect a healthy population. Even assuming that the shrimp PL being used for culture are specific pathogen free (SPF), still there are generally three major pathways which a pathogen could gain access into the culture facility, such as through land-based carriers (*e.g.* crabs, rodents, and other mammals including humans); water-borne carriers (*e.g.* wild shrimps, swimming crabs, and other wild crustaceans in the surrounding waters); and air-borne carriers (*e.g.* aquatic birds).

Constructing a biosecurity fence to enclose the culture facility could be one of the major ways of preventing land-based carriers from gaining access into the grow-out facility, as frogs, rodents, cats, dogs could be prevented entry, and the spread of viral materials by low flying birds hunting for food at night, could also be avoided. The fence would also serve as a barrier to prevent contaminated persons or materials from getting into the culture areas. Only a single access point for entry and exit should therefore be designated and provided with decontamination facilities. In this way, production personnel and equipment should be properly disinfected and sanitized before entering the fenced grow-out facility. Bigger areas may have to be divided into culture zones, so that personnel involved in each zone have to wear shirts of different colors to identify them with their respective culture zones and are not allowed to go to other zones. Individuals not involved in the culture process should be strongly discouraged from entering the "clean zone" (the area after decontamination outside of the culture zones). Persons wearing anything wet or carrying any wet item(s) into the farm should be strictly denied entry. A crab fence 30 cm in height constructed along the dikes would prevent the entry of wild crabs that bring pathogens into the ponds. Any form of crustacean-based food whether cooked and especially raw should be strictly prohibited in the culture facility.

Adoption of the ecosystem approach to management in extensive culture systems

Small-scale shrimp farmers usually have big ponds (> 1.0 ha), located close to each other, and do not have the provisions for reservoirs or settling ponds, but having the same water receiving and source, and sharing water source with other ponds. These farms are more at risk to EMS than those into semi-intensive/intensive system that are usually corporate-owned and have the financial capacity to implement innovations. One approach in establishing the preventive measures against EMS in this type of system is through the ecosystem approach to aquaculture management (EAAM), an approach which is based on the FAO Code of Conduct for Responsible Fisheries, and is especially relevant when aquaculture takes place in common properties where water resources are shared. The first step in the EAAM to prevent diseases is to identify the aquaculture management area (AMA) consisting of the aquaculture zone for aquaculture management or selected farms/ponds within the zone that are grouped/clustered, and choose the design of aquaculture management to be practiced. Aquaculture zones could include a hydrological system that encompasses part of or an entire catchment area from the source to water body that will receive the farm effluents including those from pond sites that consist of different clusters of ponds/farms (FAO and World Bank, 2015).

Since all farms in the AMA contribute to nutrient loading as well as to the possible spread of diseases and other impacts of aquaculture, a form of collective management would be necessary, but should be agreed upon by the aquaculture operators, especially with respect to certain management practices that could minimize the overall impact of their collective culture activities. Farms/ponds in the AMA should therefore establish their respective management plans but the aquaculture activities should be implemented simultaneously. This means that stocking the ponds should be done at the same time using shrimp PL obtained from the same source, water change is synchronized, the same feed and feeding management is adopted, and harvesting of the stock should be done at the same time. This process protects the environment and reduces the risk of EMS. Execution of the plan is monitored to allow for review and adoption of possible changes or adjustments, especially in the biosecurity, social, and environmental measures, as deemed necessary.

Intensified adoption of other established preventive measures

Brackishwater deepwell would be an ideal source of culture water as this is truly pathogen-free. In case surface water needs to be used for culture, a reservoir is needed to hold the water for conditioning prior to its use in the pond. Water needs to pass through fine-mesh filters before being allowed to flow into the ponds to prevent the entry of water-borne carriers. Each pond will have its own set of culture paraphernalia, *e.g.* secchi disk, feeding boats, sampling bottles, to avoid contamination. Field equipment used in monitoring pond water parameters should be appropriately disinfected between usages.

Birds have become a major concern in shrimp farming because of their ability to move from farm to farm, and have the potential to spread viral particles much faster because of their habit of feeding on weak and lethargic shrimps from nearby ponds that have been compromised with diseases. Bird-scare devices, especially UV resistant nets and lines have proven effective in preventing the entry of birds into the ponds to hunt for food. Trees that act as bird shelters inside the grow-out facility should be removed. Other unnecessary vertical structures inside the farm that birds could use to roost should be minimized or removed.

The use of high-density polyethylene (HDPE) liners has also allowed for faster crop turnovers and lesser dike maintenance between production cycles. HDPE-lined ponds have lesser amount of sludge accumulation in the pond bottom because of the protection it offers against scouring of the pond bottom and dikes. As such there will be less substrate material for pathogen growth in the pond bottom resulting in a better pond culture environment for the shrimps. Nonetheless, for the effective implementation of this program, a regular audit of existing biosecurity protocols should be performed independently every week (or as often as practically necessary) to prevent any breach in the farm's defence against diseases. This will help assure that all biosecurity protocols and procedures are properly followed and implemented.

Conclusion and Way Forward

Overall, regular monitoring of water parameters, pond bottom, and cultured shrimps should be implemented. Shrimp should be observed closely for abnormality in their swimming behavior, eating habit, and appearance. One of the first signs of disease is loss of appetite and abnormal swimming behavior. Weak shrimps should be immediately removed while shrimp samples should be sent to a diagnostic laboratory for early detection of disease so that preventive or control measures can be promptly implemented. Season of stocking shrimp PL in ponds is a risk factor for both AHPND and WSSV. Ironically, while the cold months reduce the risk due to AHPND, it increases the risk due to WSSV. To minimize mortality due to EMS, the quality of PL for stocking, as well as the quality of water and pond bottom conditions which are very crucial, should therefore be in good conditions.

Prevalent, emerging and re-emerging diseases like EMS will continue to exist. The techniques developed, improved and promoted through the JTF-funded projects will help farmers from SEAFDEC Member Countries that are into semi-intensive/intensive shrimp culture, to prevent or mitigate the effects of diseases. Farms practicing the developed techniques will have high economic returns and have high chances of getting a Certification provided that the other requirements like documentation are complied with. Promotion and proper implementation of the techniques should be strengthened. Demonstration ponds that strictly follow the GAPs and biosecurity measures should be identified and designated, to convince the other farmers that disease problems in aquaculture could be overcome through the implementation of proper farm management. The success of these demonstration ponds could then be disseminated to the stakeholders through lectures, on-farm trainings, and enhanced information, education and communication campaigns.

Small-scale farmers comprise the majority of the stakeholders, and usually have low production due to the existing set-up: adjacent to each other, no reservoir nor settling ponds, having the same water receiving and source, and sharing the same water source with the other ponds. Although the situation looks bleak for improved production and export of their shrimp produce, there is still hope for improvement provided the adoption of the EAAM concept is seriously taken up. The assistance of organizations, like FAO could also be engaged, as their expertise and with the collective effort of stakeholders, the aquaculture area management of clustered ponds could be promoted, as this concept provides the bright future in shrimp production and leads to improved performance of the ponds and enhanced eligibility for Certification as a group. At the initial state however, the EAAM concept could be applied in one area only to show to small-scale farmers that it is efficient, but the success of EAAM in an AMA would encourage the other farmers to form clusters.

Aquaculture recirculating system and use of constructed wetlands could also improve shrimp production and livelihood of small-scale farmers. Projects on the identification of aquatic organisms that have high economic value and the ability to improve water quality of pond effluent that can be used in the aquaculture recirculating system and constructed wetlands are worthy of funding. Both aquaculture recirculating system and constructed wetlands can be integrated in the culture of shrimp using semi-intensive/intensive systems and in the AMA.

The success of any form of farm management, such as AMA management, EAAM and other management schemes mentioned above, and the promotion of any developed technique would depend on the cooperation among stakeholders, and on the awareness through capacity building of the key industry players. This will pave the way to the rise of the *P. monodon* industry to the glory where it used to be.

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