Proceedings of the
International Workshop on the Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia
25-27 June 2019, Iloilo City, Philippines

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December 2021

ISBN 978-971-9931-10-2 (Print)
ISBN 978-971-9931-11-9 (PDF)

Published and printed by:
Southeast Asian Fisheries Development Center
Aquaculture Department
Tigbauan, Iloilo, Philippines

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SEAFDEC Aquaculture Department Library Cataloging-in-Publication Data

International Workshop on the Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia (2019 : Iloilo City, Philippines).

Promotion of sustainable aquaculture, aquatic animal health, and resource enhancement in Southeast Asia : proceedings of the international workshop on the promotion of sustainable aquaculture, aquatic animal health, and resource enhancement in Southeast Asia, 25–27 June 2019, Iloilo City, Philippines / Frolan A. Aya, Leobert D. de la Peña, Nerissa D. Salayo, Eleonor A. Tendencia. -- Tigbauan, Iloilo, Philippines : Aquaculture Dept., Southeast Asian Fisheries Development Center, 2021, ©2021.

xi, 288 pages : color maps, color illustrations.

Includes bibliographical references.

1. Sustainable aquaculture--Southeast Asia--Congresses. 2. Fishes--Diseases--Southeast Asia--Congresses. 3. Aquatic resources--Southeast Asia--Management--Congresses. I. Aya, Frolan A., editor. 2. de la Peña, Leobert D., editor. 3. Salayo, Nerissa D., editor. 4. Tendencia, Eleonor A., editor. I. SEAFDEC. Aquaculture Department. II. Government of Japan-Trust Fund. III. Title.

SH 136 .S88 I58 2019 DLS2021-02

Foreword

Food from the aquatic environment is a major source of protein for innumerable groups of people around the world. For millenia, the oceans, seas, rivers, and lakes provided mankind with nutritious and affordable seafood. However, the accelerating growth in the human population has increasingly strained these natural resources. Since the 1990s, global catch from wild fisheries has stagnated due to destructive fishing practices, overharvesting, and other factors. Aquaculture filled the gap between the ever-rising demand for aquatic protein and the limited wild supply. In 2014, over half of the reported fish and shellfish consumed by man was produced by aquaculture.

Aquaculture is expected to continue growing indefinitely along with population growth and increased consumptin of seafood. However, the prolifieration and intensification of aquaculture carries its own risks and challenges. Its rapid growth has already led to the degradation of culture sites, destruction of ecosystems, spread of aquatic diseases, among others.

In Southeast Asia, the aquaculture sector has been growing steadily, netting 13 million tons of produce in 2018. On the same year, four of the top-ten aquaculture producing countries were from the region. To sustain the industry and safeguard the livelihood of millions who depend on aquaculture, systems must be in place to regulate the use of limited resources and manage aquatic diseases that wreak havoc on farms worldwide. To benefit the natural aquatic environment, aquaculture must also be harnessed in stock enhancement activities that rehabilitate depleted wild stocks.

Towards these goals, the international workshop on the "Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia" (SARSEA) was organized with support from the Government of Japan Trust Fund (GOJ-TF). The event, held 25–27 June 2019 in Iloilo City, Philippines, gathered experts from SEAFDEC member countries to document and accelerate the exchange of information on the different initiatives in the region to push for more sustainable and responsible aquaculture practices. The workshop also served to identify gaps and issues that could shape the future research directions of SEAFDEC/AQD and other agencies in the region.

This publication is a product of the workshop and compiles information from the thirty presentations made during the event. Reports from member countries, invited experts, and SEAFDEC/AQD projects supported by the GOJ-TF on the topics of sustainable aquaculture, resource enhancement, and aquatic animal health are bared in these pages. The workshop summary, synthesizing the key points raised during the workshop, should be a valuable reference on the broad challenges we face, as well as the solutions and actions that must be done.

We hope that this publication achieves a wide readership as it is outlines the framework through which SEAFDEC member countries may work together towards food security through sustainable aquaculture.

DAN D. BALIAO
Chief, SEAFDEC/AQD

Message

As the world's growing population increases the demand for food, the supply of protein sources, in particular, has become a major issue. In this situation, aquatic food plays an important role as a source of protein, and its production has increased year by year. However, capture fisheries production has been stagnant since the mid-1990s due to environmental changes and overfishing. This has led to a rapid rise in aquaculture production in recent years to meet the ever-increasing demand for aquatic food. The development of the aquaculture industry in Southeast Asia has been particularly remarkable, accounting for more than 90 % of the world's aquaculture production. However, the vigorous and rapid growth of the aquaculture industry has caused many problems such as destruction of ecosystems, reduction of biodiversity, spread of diseases, and abandonment of aquaculture farms, all of which have led to social conflicts of interest. The abandonment of farms and the spread of disease, in particular, have temporarily brought the aquaculture industry to critical states. Therefore, the Japan Trust Fund Phase I (JTF6-1) addressed the following six issues in order to develop practical measures to deal with the environmental and social problems arising from their aquaculture activities: 1) Establishment of environment-friendly and responsible aquaculture technology, 2) Promotion of community-based production and resource enhancement of high-value aquatic resources, 3) Development and acceleration of rapid and effective fish and shrimp health management, 4) Enhancement of efficacy of vaccine treatment in tropical cultured species, 5) Establishment of protective measures against persistent and emerging parasitic diseases of tropical fish, and 6) Epidemiology of the Early Mortality Syndrome (EMS) /Acute Hepatopancreatic Necrotic Disease (AHPND).

The main objectives of the international workshop on the promotion of "Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia" (SARSEA) are to share the results of the JTF project conducted in AQD, to present recommendations for addressing regional aquaculture issues through the country reports, and to gain understanding and support from SEAFDEC member countries for future JTF project activities. The SARSEA international workshop was held on June 25–27, 2019 in Iloilo City, Philippines. It provided an opportunity to share information on research gaps and collaborative activities among SEAFDEC member countries. These efforts have been carried over to the Japan Trust Fund Phase II (JTF6-2) project.

In order to leave a record of the problems that have occurred at aquaculture fields in the region to date, as well as the efforts that have been made to solve their problems thus far, reports on the results of the JTF project, the country reports, and the invited presentations presented at the SARSEA international workshop have been compiled and published now as the SARSEA proceedings. By re-sharing the aquaculture situation in the region, the proceedings will significantly contribute to the development of sustainable aquaculture system based on the actual conditions of aquaculture fields in the region, and to the development of technology for the control of aquatic diseases and prompt disease countermeasures. In a way, it will serve as a guidepost for the direction of the JTF project activities in the future. By then, I hope that the aquaculture industry has grown steadily as a major industry in the ASEAN region.

SAYAKA ITO, Ph. D.
Deputy Chief and GOJ Trust Fund Co-Manager

Message

Aquaculture production in Southeast Asia has grown rapidly over the last two decades. However, the rapid growth in aquaculture also brought negative impacts to our region such as: degradation of the culture sites, destruction of sensitive ecosystems, decrease in biodiversity, spread of diseases, social conflicts, natural resources management problems and other contributing factors.

In order to promote and augment regional initiatives on sustainable aquaculture, aquatic animal health and resource enhancement, and to consequently contribute to poverty alleviation, livelihood and food security, the Southeast Asian Fisheries Development Center/Aquaculture Department (SEAFDEC/AQD) has held a 3-day workshop on the "Promotion of Sustainable Aquaculture, Aquatic Animal Health and Resource Enhancement in Southeast Asia (SARSEA)" on 25–27 June 2019 in Iloilo City, Philippines.

In the workshop, we discussed the issues related to sustainable aquaculture, aquatic animal health and resource enhancement, through "Country reports," from SEAFDEC member countries, "AQD GOJ-TF6 Research Reports," which were being carried out under the Government of Japan Trust Fund 6 from 2015 to 2019, and were presented by the AQD researchers, "Special Presentations" from the Invited Resource Speakers," and "Workshop Discussion" for identification of research gaps and collaborative activities among member countries.

I believe, these discussions could update on the issues and put forward recommendations to address these issues. As a consequence, our awareness and commitment to enhance and support research and development towards wholesome and responsible production of aquatic species will be increased.

Finally, we gratefully acknowledge the Government of Japan for the main financial support.

And special thanks are also due to the organizing committee, all the participants and speakers, particularly the member country representatives and the experts, Dr. Satoshi Watanabe, Dr. Marie Antonette Juinio-Meñez, who was co-represented by Dr. Jon P. Altamirano, Dr. Arun K. Dhar and Dr. Kallaya Sritunyalucksana-Dangtip, for their kind support and attendance to the workshop.

KOH-ICHIRO MORI, Ph. D.
Former Deputy Chief and GOJ Trust Fund Co-Manager

Workshop Overview

Animal food production has been increasing steadily in recent decades in order to address the growing demand for food of the rapidly increasing world population. Southeast Asia has been a major contributor to the global aquatic food supply. However, capture production has attained saturation levels and stagnated since mid-1990s. This shows the ever growing importance of aquaculture at present and in the future. On the other hand, the rapid growth in aquaculture also brought negative impacts to our region such as degradation of the culture sites, destruction of sensitive ecosystems, decrease in biodiversity, spread of diseases, social conflicts, natural resources management problems and other contributing factors.

Majority of these repercussions affect not only the stability of culture production but also stock levels of wild aquatic species. They also preclude efforts toward food security and poverty alleviation in the region. The increasing expansion of the aquaculture industry has undeniably intensified the demand for fishmeal as one of the primary protein sources in aquaculture feeds. Due to the increasing costs of fishmeal, various alternatives for fishmeal in aquaculture have been explored and information on their nutritive value and inclusion level have been tested to ease dependence on this expensive feed source. However, there are still other locally available substitutes which can be tapped for potential use in aquaculture feed formulations. A great volume of agro-industrial wastes and by-products are generated and disposed to the environment, and options to its efficient utilization are necessary.

Among the countermeasures to address the environmental and social issues arising from fisheries and aquaculture practices, active approaches for establishment of environment-friendly culture technologies, promotion of community-based management of aquatic resources and replenishment of endangered species are becoming increasingly significant to secure the sustainable utilization and management of aquatic species in our region.

Unsustainable aquaculture practices including the irresponsible transfer of aquatic species, particularly farmed stocks that could potentially be carrying pathogens, has also contributed to the emergence of a number of infectious diseases thereby posing serious threats to the sustainability of aquaculture in our region. Viral and bacterial diseases have caused major constraints in fish and shrimp farming in most Asian countries and in the world. The continued occurrence of the most devastating viral disease, the white spot syndrome virus (WSSV), and other pathogens such as VP_{AHPND} that cause acute hepatopancreatic necrosis disease (AHPND) necessitate the establishment of domesticated shrimp stocks that are free of these pathogens. There is also the need to develop early detection of these devastating pathogens, anti-pathogen and vaccine treatments for effective management of persistent and emerging aquatic animal disease outbreaks.

In order to promote and augment regional initiatives on sustainable aquaculture, aquatic animal health and resource enhancement, and to consequently contribute to poverty alleviation, livelihood and food security, SEAFDEC Aquaculture Department held a three-day international workshop on the "Promotion of Sustainable Aquaculture, Aquatic Animal Health and Resource Enhancement in Southeast Asia (SARSEA). This workshop was funded by the Government of Japan.

Description of Activities

1. *Country Reports*
    - Status reports of SEAFDEC member countries on sustainable aquaculture, aquatic animal health and resource enhancement practices including pressing issues, gaps, possible strategies, and recommendations in their respective countries

2. *Countributed Papers on the following topics*
    - Establishment of environment-friendly, responsible aquaculture technology;
    - Promotion of community-based production and resources enhancement of high-value aquatic resources;
    - Development and acceleration of rapid and effective fish and shrimp health management;
    - Efficacy enhancement of vaccine treatment in tropical cultured species;
    - Establishment of protective measures against persistent and emerging parasitic diseases of tropical fish;
    - Identification of risk factors and develop protective measures against Early Mortality Syndrome (EMS);
    - Conduct of training, technology extension and demonstration;
    - Other matters supporting sustainable aquaculture, fish health and resource enhancement practices

3. *Workshop Discussion - "Identification of research gaps and collaborative activities among member countries"*
    - Needs and requirements of member countries to promote sustainable aquaculture, aquatic animal health and resource enhancement practices;
    - Special needs of member countries for learning procedures and methodologies for the respective practices;
    - Application of knowledge and utilization of available resources; and
    - Training needs of personnel in member countries

Outputs

At the end of the workshop, participants were updated on the issues related to sustainable aquaculture, aquatic animal health and resource enhancement, and recommendations were put forward to address these issues. Awareness and commitment to enhance and support research and development towards wholesome and responsible production of aquatic species were increased.

Acknowledgments

The editors would like to thank the following:

- Government of Japan – Trust Fund 6 for providing financial support for the conduct of the SARSEA conference and the publication of this proceedings;
- Development Communication Section especially Mr. Rex Delsar Dianala, Ms. Rossea Ledesma, and Ms. Joesyl Marie dela Cruz for copy-editing and layout;
- Ms. Micah Danielle Lojera for editing some of the country reports;
- SEAFDEC/AQD Publication Review Committee for the constructive comments and helpful suggestions

Contents

Sustainable Aquaculture & Resource Enhancement

Current Status of Sustainable Aquaculture and Resource Enhancement in Cambodia

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Abstract

Cambodia is rich in both freshwater and marine fisheries resources. Aquaculture in Cambodia has been practiced in the Great Lake (Ton Le Sap) for a long time . The culture method involves stocking of wild juvenile fish in pens or cages and feeding with trash fish. In 1994, a new aquaculture technology was introduced in the country through the Asian Institute of Technology (AIT) outreach programme.

Aquaculture development in Cambodia is part of a national policy under the National Rectangular Strategy Policies of the Government. To support the national policy, the Fisheries Administration has introduced the updated Strategic Planning Framework for Fisheries (SPFF) for 2015. Meanwhile, the National Strategic Plan for Aquaculture Development in Cambodia (NSPAD) 2016-2030 aims to meet the growing demand for fish for domestic consumption, and future investment requirements in aquaculture development.

The main aquaculture production produced from inland aquaculture accounts for nearly 90% of the total fish production. Aquaculture systems including floating cage/pen culture, earthen pond culture and integrated rice-fish culture, and other fish culture in small scale or aquaculture-based fisheries in Cambodia are practiced in over 20 provinces and cities, with less development on coastal aquaculture

Annual aquaculture production increased by an average of 20 % over the past decade, from 50,000 metric tons in 2009 to 254,048 metric tons in 2018.

Enhancing rice field fisheries productivity continues to be a priority in the Fisheries SPF, especially through Community Fish Refuges (CFRs). Rice field fisheries provides 100,000–150,000 tons per year which contributed 20–30 % of the total inland fish production.

However, knowledge about the current status of the sector is lacking. Anecdotal field observations and the few existing studies depict a sector with unsophisticated technology, low efficiency and low competitiveness against imports from neighboring countries. Limited availability of quality inputs and services is a major constraint to the growth of the aquaculture sector. Fingerling production, in particular, is insufficient and the poor quality of fingerlings produced results in very low levels of production to support the industry leading to the importation of fingerlings from neighboring countries.

Introduction

Cambodia is rich and has high diversity of freshwater fish, with more than 400 species (IFReDI, 2019). In general, fish consumption and fish supply for local and international markets mainly depend on inland fisheries; only a small portion comes from marine fisheries. The decline in inland fisheries fish stock drove the aquaculture sector to contribute to rural livelihood.

Cambodia has identified aquaculture as one of the three most important pillars of the country's fisheries development. The government's Strategic Planning Framework (SPF) for Fisheries for 2010 to 2019 considers expanding the farming of fish and other aquatic animals as "essential" given the limited capacity of natural resources to sustain the country's growing population. To support the growth of small, medium and large-scale freshwater aquaculture, government spending on aquaculture has been budgeted at more than $16 million under the 10-year framework. Recently, aquaculture extension is one of the national policies under the SPF for fisheries sector. To achieve the sectorial goal, several freshwater aquaculture systems including floating cage/pen culture, earthen pond culture and rice-fish culture, and other fish culture in small water bodies or aquaculture-based fisheries in Cambodia have been practiced in over 20 provinces and cities, with less development focused on coastal aquaculture.

The aim is to boost production from both freshwater and marine aquaculture from 360,000 tonnes in 2020, to 740,000 tonnes in 2024 (SPF, 2016). In this decade, the aquaculture sub-sector has remarkably grown while production from capture fisheries in the country decreased. Promotion of aquaculture is an important contribution to national food security as well as to the country's revenue generation.

Aquaculture systems

The average annual growth of the aquaculture sector in Cambodia is 10 % over the last 20 years and has consistently been above 18 % over the last 10 years. In 2014, nationwide production is 112,000 tonnes which includes both marine and freshwater production. Aquaculture production is projected to increase from 76,000 tonnes in 2012 to 202,000 tonnes in 2019 (Strategic Planning Framework (FiA), 2011).

Aquaculture production is localized to specific provinces within the country. The Tonle Sap and the Mekong River have cage culture. In 2014, Siem Reap, Pursat and Phnom Penh were home to 61 % of the volume of cage culture nationwide. The lower floodplains have pond culture (79 % of the total pond area). A growing aquaculture sector is based around the outskirts of Phnom Penh. Recent aquaculture development projects have increased the number of ponds in targeted provinces, although nationwide, the overall number of ponds decreased by 9 % from 2009 to 2014.

Six main production systems represent more than 99 % of the total aquaculture production in Cambodia. Freshwater cage culture dominates the sector (more than 50 % of total production), followed by small and medium-sized enterprises (22 %) and smallholder high-input ponds (18 %). Other systems such as smallholder low-input ponds, marine cage culture and rice-fish systems are of minor importance. Marine low-value fish account for 3 % of the total feed used in aquaculture, while manufactured pellets represent less than 1 %. The rest of the fish feed used in aquaculture is provided by Cambodia's inland capture fisheries.

Aquaculture in Cambodia is dependent on capture fisheries which supplies the

sector with feed and seed for most of the semi-intensive ponds and cage systems. The total value of aquaculture production in 2011 is estimated at $114 million, freshwater cage farming reached 37,000 tonnes during that year (WorldFish, 2011). In terms of production and gross revenue, pangasius and snakehead dominate, with more than $30 million generated for both species. Though marginal, marine cage production is estimated at $7 million.

Freshwater

Cambodia has many water resources, such as the Great Lake Tonle Sap, the Mekong River, the Tonle Sap River, the Bassac River and many of their tributaries. A number of these lakes are potential sites for aquaculture. Freshwater aquaculture includes culture in cages, ponds and pens in areas with abundant water resources or are irrigated. Recently, fish culture have spread throughout the country, including the upland areas. Freshwater pond culture covers a total area of 1,350 ha of earthen ponds, comprised of 39,955 ponds. Floating net-cage culture is also important and covers 12 ha, comprised of 4,224 cages (FiA, 2014). These cages are used primarily for snake head (*Channa striatus*), giant snake head (*Channa micropeltes*), silver barb (*Barbonymus gonionotus*), *Pangasius* spp. and *Mystus spp.*

Cultured species and potential species for culture

Freshwater aquaculture is more developed than marine aquaculture. Cultured fishes include both indigenous and exotic species. The major cultured species are *Pangasius* spp. (73 %) followed by giant snake head (*Channa micropeltes*) (21 %). Other species produced include *Puntius sp.*, Thai catfish (*Clarias batrachus*), marble goby (*Oxyeleotris marmorata*), *Cirrhinus* sp., red tailed tinfoil (*Barbonymus altus*) and Hoven's carp (*Leptobarbus hoeveni*).

Culture systems, techniques, feeds and feeding management

Small-scale

A number of projects that dealt with small-scale aquaculture development are implemented in collaboration with the Fisheries Administration such as the following:

1. Agriculture Productivity Improvement Project (APIP);
2. Aquaculture of Indigenous Mekong Fish Species (AIMS/MRC);
3. Asian Institute of Technology-(AIT–ARRM); and
4. NGO. Native fish species are commonly used for pond culture with *Pangasius hypophthalmus* as the major cultured species. Please see **Table 1** for the other cultured species.

Pond fertilization techniques are well understood by farmers through aquaculture extension workers in project sites in some provinces. Green water is commonly used to rear fish by applying organic fertilizers. Small-scale farmers also apply inorganic fertilizer in order to improve pond productivity. Rice bran, broken rice and waste vegetables are the most common feed ingredients used in Cambodia. These ingredients are sometimes fed directly without processing; although, a few farmers do so, depending on the availability of labour and firewood. Other feeds used by most small-scale aquaculture farmers are duckweed, termites, cassava leaves, kitchen wastes and rice wine waste. Integrated fish-farming is also practiced in some areas, such as pig-fish, duck-fish, chicken-fish, rice-fish and garden-fish etc. These

Table 1. Most common fish species in Cambodia and their respective culture method and seed source

Species name	Farming system	Source of seed	Production volume
Native			
Striped catfish (*Pangasius hypophthalmus*)	floating cage, pond	hatchery, wild	high
Basa fish (*Pangasius bocourti*)	floating cage	wild	high
Spot pangasius (*Pangasius larnaudii*)	floating cage	wild	low
Trey pra ke (*Pangasius conchophilus*)	floating cage	wild	low
Giant snake head (*Channa micropeltes*)	floating cage	wild	high
Snake head (*Channa striatus*)	floating cage	wild	high
Silver barb (*Barbonymus gonionotus*)	floating cage, pond, rice field	hatchery, wild	high
Hoven's carp (*Leptobarbus hoeveni*)	floating cage, pond	hatchery, wild	medium
Trey khya (*Mystus wyckiode*)	floating cage	wild	low
Marble goby (*Oxyeleotris marmorata*)	floating cage, pond	wild	low
Snakeskin gourami (*Trichogaster pectoralis*)	pond, rice field	hatchery, wild	low
Red tailed tinfoil (*Barbonymus altus*)	pond, rice field	hatchery, wild	low
Exotic			
Nile tilapia (*Oreochromis niloticus*)	floating cage, rice field, pond	hatchery	medium
Silver carp (*Hypothalmichtys molitrix*)	pond	hatchery	medium
Common carp (*Cyprinus carpio*)	rice field, pond	hatchery	medium
Bighead carp (*Hypophthalmichthys nobilis*)	pond	hatchery	low
Grass carp (*Ctenopharyngodon idellus*)	pond	hatchery	low
Mrigal (*Cirrihinus mrigal*)	pond, rice field	hatchery	low
Hybrid catfish	pond	hatchery	high
African catfish (*Clarias gariepinus*)	pond	hatchery	high

Source: FIA, 2014

practices effectively reduce feed cost and increase fish production. Feed represents more than 70 % of the total operational cost and is mainly from small sized or low value fish which is between 60 % to 100 % of the total feed used depending on feeding strategies adopted by different farmers (So *et al*., 2005). During the dry season (October to May), the most important source of feed is freshwater small sized or low value fish, while more marine small sized or trash fish are used during the rainy season (June to September).

Commercial-scale

Cage culture is the most prevalent aquaculture practice in Cambodia and commonly practised in rivers and streams in provinces bordering the Great Lake. The major fish species for cage culture are river catfish (*Pangasius hypophthalmus*, *P.*

bocourti and *P. larnaudii*) and snakehead (*Channa micropeltes*). The river catfish, *P. hypophthalmus* is the dominant species for cage and pond culture. Fish production from cage culture systems is much higher than from pond culture. Commercial-scale cage culture in Cambodia contributed about 70 % of the total aquaculture production.

The main feed for pangasid catfish and snakehead is low-value fish (such as small cyprinids), which are available during peak season of fish catch, particularly from November to January. Small cyprinids are caught by 'dai lot' (bag net fishing) along the Tonle Sap River. Availability of feed ingredients varies both regionally and seasonally. During the peak of the fish catch, cultured fish are overfed, due to an abundance of low-value fish. After the peak period, fish are fed cooked rice bran mixed with 10–20 % of dry fish, depending on availability. Aquaculturists rarely use commercial pellets to feed fish because its use has not yet been widely disseminated in Cambodia. Furthermore, commercial feeds are imported and commands higher price than other feed types. Commercial fish pellets are not produced locally due to its high production cost and low market demand. However, Cambodia produces feed pellets for livestock and poultry which some rich farmers also use to feed fish, but it is not profitable at present.

The estimated feed conversion ratio (FCR) for pangasid catfish around Phnom Penh municipality during 1994–1995 season are as follows:

- fed only rice bran, FCR = 1:4 to 1:4.5
- fed rice bran mixed with low value fish and dried fish, FCR = 1:3 to 1:3.5

Production, consumer demand, cost of production, profitability

FiA statistics show that total aquaculture production reached 39,025 tonnes in 2008, representing 11 % of total inland fishery production. Inland fishery production came from cage culture. There are approx. 4,500 cages that can be found along the Mekong River (33 %), Tonle Sap River (17 %), Bassac River (7 %), and in the Tonle Sap Lake (43 %) which contributes 70-80% of the country's aquaculture production (So and Haing, 2007; Viseth and Pengbun, 2005). The rest comes from pond-based production systems. The number of ponds used for aquaculture increased from 3,455 in 1993 to 56,234 in 2009. However, the contribution of ponds to overall production remains limited because of the generally low productivity in low-input, extensive homestead fish ponds (So, 2009b). Fish and seed production are centered near cities where the communication and market networks are well developed: Kandal province and Phnom Penh account for 49 % of the total aquaculture production and 57 % of the fingerling production (FiA, 2007).

Researches done

There are a few researches being carried out at the National Aquaculture Research and Development Institute (NARDI) and Bati Center. Current research is more focused on the breeding of indigenous species, with high economic value such as the giant freshwater prawn, *Himibagrus wickioides* and *Osteichilus melanopleura*; and the domestication of some species such as *Tor tambra*, *Chitala ornata*, *Catlocarpio siamensis* and *Osphronomus goramy.*

A few studies on the cage and pond culture of snakehead in Cambodia were

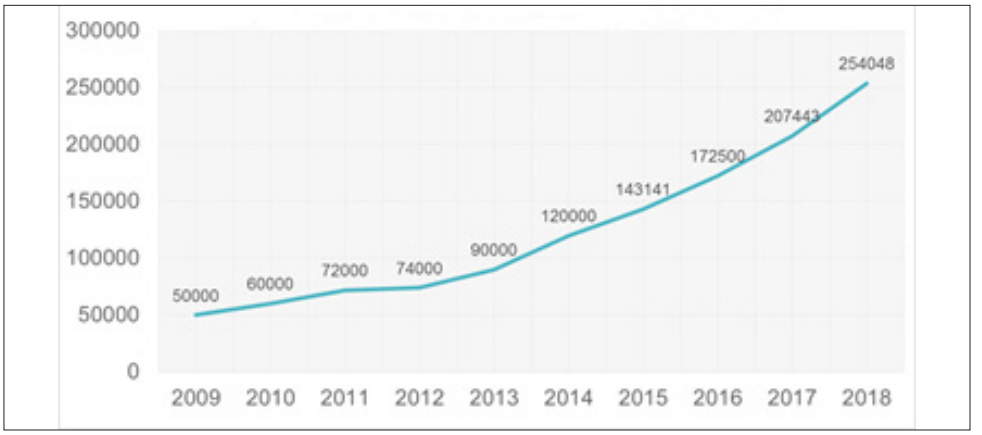

Figure 1. Annual production (in metric tons) from aquaculture sub-sector

done for cost and profitability analysis. Furthermore, factors affecting the success of the business were identified which can be the basis for the promulgation of policies which aim at promoting sustainable development of snakehead aquaculture practices in Cambodia.

Research gaps

The main extension priorities recommended for Cambodia are identified as follows:

- Research on breeding techniques for some other indigenous species, with high economic value;
- Building on the capacity of breeding selection and genetic conservation;
- Research on fish diseases and applied vaccination in aquaculture;
- District and provincial level government capacity building – to enable local staff to carry out breeding, feed and feeding trials and extension;
- Develop better alternatives for small-scale credit schemes – to address the lack of available credit for farming communities;
- Assistance to develop a fish-feed enterprise in Vientiane – to encourage development of a fish-feed center in Vientiane ensuring local ownership and operation; and
- Farmer networks – research into effective ways to develop farmer-to-farmer feed extension networks, similar to other networks developed by AIT and UNDP/FAO.

Problems encountered

Inland aquaculture in Cambodia is still faced with issues and problems regarding breeding and nursery techniques. In addition, the capacity building of fisheries officer is really needed.

Breeding of several indigenous species such as *Barbonymus altus* and *Barbonymus gonionotus* are quite successful but needs to be improved for other species. There are two types of feed, fresh and homemade feed, that farmers use for intensive and semi-intensive culture systems. Fresh feed includes trash fish, fish byproducts, poultry by-products, kitchen waste etc. These

fresh feed materials are dumped directly into the fishpond, causing deterioration of water quality. Moreover, without proper storage, fresh feed spoils easily and its quality can deteriorate. This increases the risks of disease occurrence. This problem is often found in small farms which lack cold storage facilities to keep raw materials.

Management practices

In general, aquaculture is under the management of Cambodian Fisheries Law. Exotic species are banned for culture in natural water bodies. In recent decades, giant snakehead culture is commonly found in areas with high supply of low value fish such as from Tonle Sap Lake, Bassac and Mekong Rivers. The conflict in the use of low value fish as feed to giant snakehead and other cultured species and for human food consumption was recognized; and thus, farm-raised snakehead was banned in 2004. Recently, the Fisheries Administration issued a proclamation on management measures for sustainable snakehead fish farming to the Ministry of Agriculture lifting the ban on farm-raised snakehead.

Monitoring of aquaculture systems is under the Fisheries Administration, directly under the Department of Aquaculture Development. Recently, Good Aquaculture Practice guideline is introduced and disseminated to all stakeholders. The National Aquaculture Development Strategy (NADS) developed a roadmap for the aquaculture sub-sector

Sustainability

To support the sustainability of aquaculture in Cambodia, policies, strategic plans for the fisheries sector and national aquaculture development strategy (NADS) were developed and amended. The goal of NADS is a commercially viable and environmentally sustainable aquaculture sector contributing to food security and nutrition, socioeconomic development, GDP and export earnings. Seven strategies to guide the sustainable and development of aquaculture in Cambodia:

- To increase access to high quality of seed for a range of species in demand in local, regional and global markets;
- To ensure widespread availability of sustainably sourced, reasonably priced, high quality feed suitable for a range of species;
- To increase access to sufficient and consistent supplies of high quality water, and to reduce flood risk;
- To improve efficiency, profitability and sustainability of aquaculture production through increased knowledge, skill and organization;
- To maintain environmental quality and minimize loss from the disease;
- To increase quality and value of production;
- To facilitate access to credit as appreciate to the need, potential and risk associated with aquaculture development.

Brackishwater

The contribution of brackishwater fishery to socio-economy is not well studied and documented, particularly the aquaculture activities, production, consumer demand, research and problem encountered is not studied and presented in this system.

Mariculture

Cambodia's coastal zone, located on the south-west edge of the country, extends 435 km, and includes 85,100 ha of mangrove forests in three provinces: Koh Kong, Sihanouk Ville and Kompot (Landsat, 1994). Marine aquaculture production (mostly snapper and grouper) is projected to increase by 8 percent per year between 2009 (est. 2,880 tonnes) and 2030 (est. 15,000 tonnes) (WorldFish, 2011).

Cultured species and potential species for culture

The common marine crustaceans cultured are shrimp and mud crab. The common cultured finfish are sea bass, snapper, grouper and cobia. *Eucheuma cottonii* was cultured in Kampot province by a Malaysian company in the mid 2000s, with production reaching 18,500 tons in 2005. However, no production of farmed seaweed has been reported since 2006 (Lan, 2015). Marine species being cultured in Cambodia including the culture method and source of seeds is presented in **Table 2**.

Culture systems, techniques, feeds and feeding management

Finfish and crustacean farming are mostly semi-intensive usually done in cages, ponds and pens. Extensive culture of bivalve molluscs is done in the coastal areas. Marine aquaculture constitutes 218 ha of earthen ponds (10,232 ponds), 1 571 ha of pens (292 pens) and 14 ha of floating net-cages (1,898 cages) (FiA, 2014). Marine aquaculture practices are as follows:

(1) marine finfish culture in floating net-cages,

(2) marine finfish culture in ponds,

(3) mud crab culture in ponds. Feed is solely locally sourced trash fish.

Production, consumer demand, cost of production, profitability

Aquaculture production increased from 50,000 tons in 2009 and to 70,000 tons in 2012; from 120,000 tons in 2014 to 250,000 tons in 2018 as seen in F**igure 2.** As capture fisheries decline and the local demand for fish is high at 62.5 kg/person/year, aquaculture sector became the most important source of food in Cambodia. Aquaculture production is targeted to be 740,000 tons by 2024

Researches done

Applied aquaculture research is carried out by the National Research and Aquaculture Development Institute (NARDI) of the Fisheries Administration (FiA), Prek Leap

Table 2. Marine fish species cultured in Cambodia

English name	Culture method	Seeds Source
1. Sea bass (*Lates calcarifer*)	Pond and net cage	Hachery and import
2. Snapper (*Lutjanus* spp.)	Net cage	Import and wild
3. Grouper	Net cage	Import and wild
4. Shrimp	Pond	Wild and import
5. Cobia	Net cage	Wild and import
6. Mud Crab (*Scylla serrata*)	Pond	Wild
7. Seaweed (*E. cottonii*)	Raft	Import & local farmer

Agriculture College, National Agriculture College Kampong Cham and the Royal University of Agriculture. The NARDI has two research Divisions. Most of the applied research undertaken is concentrated on breeding techniques, broodstock improvement, seed production, nutrition, fish diseases and production technologies in ponds, cages and rice paddy fields. Most of the researches are on grouper and mud crab.

Research Gaps

One of the research gap is on selective breeding and genetic conservation in order to ensure that the produced fingerling from government hatchery station are good in quality.

Problems encountered

The problems encountered in Cambodian aquaculture are as follows:

- Deficiency of human resource, both skill and expertise on aquaculture and lack of technical assistant for local fish growth out famer and local hatchery;
- Inadequate and unreliable supply of good quality seed;
- Deficiency of capital, fund or credit for aquaculture investment;
- Adequate knowledge of aquaculture technology; inadequate manpower for aquaculture extension service; and climate and;
- Most aquaculture activities are small scale, lack of infrastructure for aquaculture practice and depend on rain feed;
- Chronic disease problems (up to 50 % losses), which seem to be endemic throughout the region.

Management practices

Cambodian fisheries is governed by the Law on Fisheries and its regulations, issued on 21 May 2006 and is currently being updated. In the updated Law on Fisheries, Aquaculture regulations are included in Chapter 10, under Aquaculture Management; some articles are related to aquaculture activities in both marine and freshwater (Article 53). The Fisheries Administration (FiA) is the principal government agency responsible for managing and developing fisheries and aquaculture. Its mandate and structure are set out in the Sub-decree. The new Law on Fisheries is divided into 17 chapters and 109 articles covering definition, exploitation of freshwater and marine fisheries, aquaculture and the processing of freshwater and marine fishery products, competent authorities for solving fishery violation, penalties and the final order.

Sustainability

Sustainability for mariculture is the same as for freshwater.

Resource enhancement

Stock Enhancement

Stock enhancement programs use seedstock produced for aquaculture purposes and from captive breeding techniques. Techniques to breed fish in captivity are developed for some species and thus the availability of hatchery-produced juveniles for stocking.

Stocking programs have been subjected to substantial criticism due to perceived impact of hatchery-bred fish on genetic

structure and fitness of wild stocks, transfer of disease and their effects on other aquatic species and the environment (no research done on this case).

Country present situation

Annual production of inland and marine capture fisheries

The fisheries sector officially accounts for about 12 % of GDP, and provides most Cambodians with their key source of animal protein, calcium and vitamin A. Cambodian fisheries products are also exported to many other countries, providing much-needed revenue.

Species stocked, scale of stocking

Stocking of indigenous species is usually done during the national fish day together with activities related to stock enhancement and community fish refuge. During the national fish day some indigenous species are stocked in large reservoirs to enhance wild brood-stock as well as increase public awareness on fisheries conservation. Species stocked in reservoir during the national fish day are in **Table 3**.

Source of seeds

Stock enhancement activity for marine species is depended on the supply of fingerlings from government hatcheries and research centers. For the stock enhancement at community fish refuge, stocks are collected wild brood-stock.

Release strategies, site selection, enclosures, monitoring

The release strategy, site selection and monitoring of fish released during the national fish day is not well established as this is just to raise public awareness, not to enhance wild stock. Stock enhancement during national fish day is done in large reservoir that interests the public. The release strategy, site selection and monitoring process for community fish refuge is more scientific. Through an advocacy campaign called "One Commune, One Community Fish Refuge" and through the legal framework of Community Fisheries (CFi), the government of Cambodia has been promoting Community Fish Refuges (CFRs) (Joffre *et al*., 2012). Joffre *et al*., 2012 describes three steps in establishing a CFR: site selection, institutional arrangements for CFR managements, and local implementation of CFR activities. Identification of appropriate sites is the most important. The CFR site should be selected in consultation with local communities. Specifically, stakeholders such as Commune Chiefs, Commune Councillors, police officers and village heads who are knowledgeable about the local context should be consulted. The site selection should also consider hydrological, socioeconomic and governance factors for CFRs' sustainability. The site should have access to year-round water supply, infrastructures for water management and enough flood level to allow fish migration. CFRs are likely to be successful in areas where there are community-managed ponds and where community members and local authorities are committed to work in harmony. The second step is the formation of CFR Committee and development of rules and regulations for managing the CFR. The third and final step includes the preparation of ponds and filling them with required volume and quality of water, securing fish pathways and plant cover. Then, brood stock and fingerlings are released. Common fish species for the CFR include snakehead (*Channa striata*), catfish (*Clarias batrachus*), climbing perch (*Anabas testudineus*) and the hatchery-raised silver barb fingerlings (*Barboides gonionotus*).

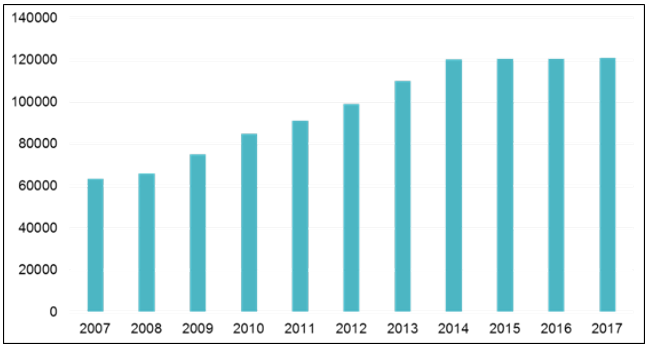

Figure 2. Annual production of aquaculture from 2009 to 2017

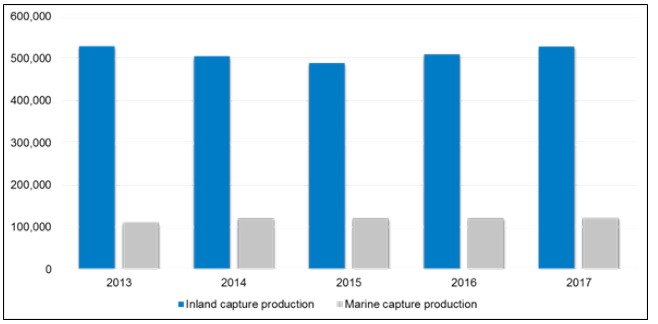

Figure 3. Annual production of inland and marine capture fisheries, Cambodia, 2013-2017

Table 3. Species stocked during the national fish day

Species	Freshwater species	Marine Species
1	*Cirrhinus microlepis*	*Epinephelus* sp.
2	*Trichohodus pectoralis*	*Thalamita crenata*
3	*Macrobrachium rosenbergii*	-
4	*Anabas testudineus*	-
5	*Channa striata*	-
6	*Barbonymus altus*	-
7	*Catlocarpio siamensis*	-
8	*Barbonymus gonionotus*	-

FiA plans to develop one well-functioning CFR in every 1200 communes by 2019 (FIA, 2018). Despite these interventions ongoing for years, there have been no systematic studies on rice field fisheries, particularly about the socioeconomic status of CFR users and their participation in the CFR process.

Management of stocked populations (population, genetics, protection, regulation)

Management of the stock population is under the Cambodian Law on Fisheries. In order to enhance wild stock, the chosen location such as deep pool in the

upper part of the Mekong is converted into a conservatory for broodstock fish protection and for freshwater dolphin. Global significant habitat such as Ramsar site located along the Mekong and Wetland area under UNESCO, at Prek Toal (Tonle Sap Lake) is banned for any fishing activities.

The management of stocked population during the national fish day is limited, whereas, the community fish refuge is more effectively manage by a committee. The protection and regulation of community fish refuge is generally followed and regulated by by-laws, internal regulations and rules implemented by the local community. Stocked fish are mature and from the wild. After stocking, fishing of the wild broodstock in the reservoir is prohibited; broodstocks can be fished during the rainy season when fish swim away from the reservoir.

References

FiA. 2018. The Strategic Planning Framework for Fisheries: 2010–2019; Fisheries Administration (FiA): Phnom Penh, Cambodia, 2010; Volume 1. Available online: http://faolex.fao.org/docs/pdf/cam143042.pdf (accessed on17 September 2018).

FiA. 2009. The Department of Fisheries, Annual reports of the Fisheries sector in Cambodia.

FiA. 2014. The Department of Fisheries, Annual reports of the Fisheries sector in Cambodia.

FAO. 2011a. The State of Food Insecurity in the World. Rome: FAO.

FAO. 2011b. FAO Yearbook. Fishery and Aquaculture Statistics. Rome: FAO.

FAO. 2012a. The State of World Fisheries and Aquaculture 2010 . Rome: FAO.

FAO–FISHSTAT. 2012. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. FishstatJ, a Tool for Fishery Statistical Analysis, Release 2.0.0. Global Capture Fisheries Production 1950–2010, Global Aquaculture Production 1950–2010, Global Commodities Production and Trade 1978–2009 . Rome: FAO.

Kawarazuka, N. & B´en´e, C. 2011. The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. Public Health Nutrition 14, 1927–1938. doi: 10.1017/S1368980011000814.

Joffre, O.; Kosal, M.; Kura, Y.; Sereywath, P.; Thuok, N. 2012. Community Fish Refuges in Cambodia–Lessons Learned; The WorldFish Center: Phnom Penh, Cambodia.

Ouch Lan. 2015. Current status of sustainable aquaculture in Cambodia.SEAFDEC, July 20, 2016 at 5:08 PM CST, IP Address: 122.55.1.77.

So N. 2009b. Impact Survey of the Freshwater Aquaculture Improvement and Extension Project (FAIEX). JICA/FAIEX, Phnom Penh 117 pp.

Viseth H. and Pengbun N. 2005. An overview of aquaculture in Cambodia. Department of Fisheries, Phnom Penh, Cambodia. 48 pp.

UNHRC. 2012. The Rights to Fish for Food. New York, NY: United Nations Human Rights Commission. Available at http://www.srfood.org/images/stories/pdf/officialreports/ 20121030 _ fish_ en.pdf/.

REPORT 4 Fauna and flora diversity studies in Botum Sakor National Park, Cambodia April 2005 – September 2009 Frontier Cambodia, January 2010. Society for Environmental Exploration 2010.

Sustainable Aquaculture Development in Indonesia

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Abstract

Despite the abundance of potential marine resources, including fisheries, Indonesia is still struggling with several issues, particularly in the aquaculture sector. Environmental issues, aquaculture feeds, fish diseases, and exceeding carrying capacity are some of the many challenges that Indonesia must face these days. The Indonesian government through the Directorate General of Aquaculture (DGA), Ministry of Marine Affairs and Fisheries (MMAF), however, has undertaken efforts to overcome the challenges and at the same time ensuring the sustainability of the sector. Sovereign, competitive, and sustainable aquaculture development policies have been implemented through three main aspects of development: 1) production technology, 2) socioeconomics, and 3) natural resources.

Harmonizing and simplifying regulations to encourage investments, interconnecting business chain from downstream to upstream in the industry and strengthening product competitiveness through IndoGAP (Good Aquaculture Practices) implementation are among the steps taken by the government. However, among the efforts to overcome the existing challenges and gaps, Indonesia continues to strive to develop its potential to make Indonesian fish farmers more prosperous and independent in the best possible way.

Keywords: sustainable, aquaculture development, Indonesia

Introduction

Indonesia is a country with abundant natural resources that produces various agricultural, livestock, and fishery products. With the vast area of the Indonesian ocean, the country can produce a large amount of fish and fishery products by capturing fish from the sea. Fishing has been carried out for many years and has become the main livelihood of Indonesian people in several locations, especially in coastal areas. In addition, fishing is becoming important because fishery products are alternative sources of protein and substitute for livestock products such as chicken and beef. However, along with the development of the fishing industry and the growing needs and demands for fish and fishery products, fishing practices are becoming increasingly

unsustainable and uncontrolled. Many fishing industries capture fish in the sea by ignoring the concept of sustainability through overfishing practices, which results in a reduction in stocks. Whereas the need for fishery products is increasing, both in terms of local and export demand. Aquaculture is gradually practiced as an alternative activity to produce fish products. Indonesia itself shows strong potential for aquaculture as the country is comprised of 16,056 islands and has a coastline of around 81,000 kilometers. From the total aquaculture potential area of 17.9 million ha, only 1.3 million is used for aquaculture, which accounts for only 7.4% (Investment Guideline for Sustainable Aquaculture in Indonesia, 2018).

Indonesia is the third-largest producer of aquaculture products in the world as its aquaculture production in 2018 recorded 14.77 metric tons, immediately following China and India (FAO, 2018; The World Bank Group, 2021). Indonesia has been practicing aquaculture many years ago, initially by many families as a backyard activity for additional income. Eventually, the sector developed rapidly and started to become a major source of household income. Aquaculture these days is an important livelihood in rural communities in Indonesia (Rimmer *et al.*, 2013). Given the abundant resources of Indonesia, aquaculture is practiced in three environments: freshwater, brackish water, and marine water. The potential land area for aquaculture is approximately 12 million ha, however, only about 325,825.11 ha is utilized. Given that there is approximately 11,797,558 ha of unutilized area, there are opportunities to develop and implement sustainable aquaculture in these areas (PEMSEA, 2019). Consequently, with the growing practice of aquaculture, more resources were used and have caused impacts on the environment. Therefore, regulations are required to control and manage the sustainability of the sector. In this paper, the existing national and international efforts to achieve a sustainable aquaculture were reviewed. This includes issuance of regulations, cooperation with international organizations, development of new aquaculture technologies, and research and development. The issues and challenges to achieve a sustainable aquaculture as well as the strategies on how to combat these issues were also enumerated.

Sustainable Aquaculture

Conducting aquaculture activities that harmonize with the maintenance of the environment and the safety of its practices is an important concern that can contribute to sustainability. These aquaculture activities include not using prohibited chemicals, not making aquaculture sites near mangroves or on coral reefs, and so on. These are the things that need to be considered and applied by all fish farming communities in Indonesia, both small-scale and industrial, in order to realize the sustainability of aquaculture in Indonesia.

The Indonesian government has made several efforts to realize the sustainability of aquaculture through the issuance of regulations, technical assistance cooperation from regional and international organizations such as FAO, SEAFDEC, NACA, etc. in order to help Indonesia implement good aquaculture practices. Sustainability in aquaculture is not just about the security of its natural resources and the stability of its business but also about how to enhance the capacity of its human resources so that they can do the business in a responsible way. FAO also mentioned that the promotion of sustainable aquaculture development requires that "enabling environments", in particular those aimed at ensuring

continuing human resource development and capacity building, are created and maintained (FAO, 2017). This was then put by FAO into FAO Code of Conduct for Responsible Fisheries which contains principles and provisions in support of sustainable aquaculture development. The Code recognizes the special requirements of developing countries, and Article 5 particularly addresses these needs, especially in the areas of financial and technical assistance, technology transfer, training, and scientific cooperation.

Environment, farmed species, and potential species for culture

Aquaculture is practiced in three environments in Indonesia. These are freshwater, brackish water, and marine water. In freshwater environment, there is a variety of cultured species. Some of the most common freshwater commodities are tilapia, common carp, catfish, pangasius catfish, and gourami. Freshwater fish are highly in demand among Indonesians. These freshwater species are also relatively affordable compared to wild-capture fish like tuna, cakalang, tenggiri, kakap, etc. Apart from the species mentioned above, the government has also encouraged local species to be developed, such as belida, toman, nilem, etc. There is also good potential for freshwater aquaculture in ponds, rice-fish farming, and in open waters such as lakes, swamps and rivers. However, aquaculture in public waters must be done in an environmentally friendly manner, productive, and in accordance with the use of open waters for other purposes. Regulation on carrying capacity is one of the parameters required to preserve environmental sustainability. Meanwhile, the potential land area suited for rice-fish farming is still very large, yet it has not been fully utilized. It is estimated that there are potential rice-fish farming areas as much as about 1.5 million ha throughout Indonesia. Rice-fish farming increases land productivity. There were more yields obtained from harvest of both paddy and fish. It also promotes a more ecosystem-friendly approach to rice farming by reducing the use of pesticides and potentially reduces urbanization and land conversion.

In brackishwater environment, commodities to be cultured are giant tiger shrimp, vannamei shrimp, milkfish, and crab. Brackishwater aquaculture production figures are also quite high, considering the products have their own markets. But when prices are compared, brackish water products still tend to be more expensive than freshwater products.

Mariculture, on the other hand, is a relatively new sub-sector in Indonesia, in contrast to freshwater and brackish water aquaculture, both of which have been practiced for centuries. Given that Indonesia is an archipelagic country comprising around 16,056 islands and has a coastline of around 81,000 kilometers, there are large areas that have considerable potential for mariculture development. However, with such vast potential, about 12,123,383 ha, so far only 328,825 ha have been utilized. The Indonesian government, therefore, has been developing mariculture to utilize the resources as well as to meet the local and export demand for marine products. In marine culture, Indonesia produces some high-value products such as grouper, seabass, seaweed, barramundi, pompano, grouper, and marine ornamental fish. The other major commodity groups are marine finfish and pearl oysters. Potential commodities being developed for mariculture in Indonesia include abalone, sea cucumber, and spiny lobsters.

Aquaculture systems, techniques, and feeding management

Classification of aquaculture practiced in Indonesia based on the number of the species are monoculture, polyculture, and Integrated Multi-trophic Aquaculture (IMTA) in land-based and water-based systems. Each aquaculture environment is using a different system, depending on the scale of the business and the capacity of the environment. Aquaculture is practiced in an extensive (traditional), semi-intensive, and intensive system. Big industries use super-intensive systems to increase production. Freshwater aquaculture is mostly practiced in semi-intensive ponds, floating cages in open waters, and rice-fish farming system on paddy fields. To boost the productivity of freshwater aquaculture, the government has developed technologies to increase production and improve product quality, such as biofloc technology for catfish, mini-recirculation aquaculture system (RAS), and rice-fish farming for tilapia. To address the problem of dependency on imported raw materials as well as to decrease feed cost, the government has made a program of feed self-sufficiency (GERPARI) that focuses on freshwater aquaculture/ commodities.

Brackishwater aquaculture is usually practiced in locations close to brackish or saltwater sources, for example near a beach or estuary to facilitate the filling and replacement of pond water. For locations along the estuary, cage ponds can be made as a practical fisheries investment. As for other ways, earthen ponds, cement ponds, tarpaulin ponds, and plastic ponds can also be made as needed.

Marine culture is one of the fisheries businesses conducted by developing resources in open and closed systems for the culture of marine organisms, either in open sea, closed sea, tanks, ponds, or waterways filled with seawater. A site for marine aquaculture must have certain natural facilities, especially a very adequate water supply, with suitable temperature, salinity, and fertility. Marine aquaculture can be practiced both off-shore and on-shore using cages.

Several technology packages of aquaculture research and engineering are used by fish farmers to improve the quantity, quality, and productivity of aquaculture efficiently. Broodstock center program has produced high-quality breeders from various species and high-quality seeds. Success in the production of vaccines, probiotics, and immunostimulants for fish/shrimp, issuance of Indonesian National Standards in aquaculture, as well as the distribution of fish health and environmental laboratory kits primarily for the early detection of fish diseases owned by the government, private sector and universities have enhanced the growth of aquaculture business effort.

One of the examples of government support towards the development of aquaculture technology that is currently underway is the application of shrimp ponds clustering in order to develop principles of responsible, environment-friendly, and sustainable aquaculture that is managed based on its carrying capacity. Through clustering, the technical side of the application of biosecurity will be more stringent, and the management of aquaculture waste and conservation-based cultivation will be more effective. These will directly prevent the occurrence of pests and shrimp diseases and minimize the impact of aquaculture on existing ecosystems. It also ensures that shrimp products can be highly competitive in the global market.

Production, consumer demand, cost of production, profitability

Aquaculture production in Indonesia is quite significant. It reached up to 16,114,991 tonnes in 2017. It comprised freshwater culture at 3,531,686 tonnes (22%), brackishwater culture at 2,698,635 tonnes (17%), and marine culture at 9,884,670 tonnes (61%) (PEMSEA, 2019).

From 2010-2014, the trend of aquaculture production has been increasing with an average growth rate of 23.73%. The trend of aquaculture production is presented in **Figure 1.**

Consequently, the contribution of fisheries to the national economy of Indonesia showed an increase from 2014–2018. The share of fisheries to the country's GDP in 2014 is 2.32% and increased to 2.60% in 2018 (**Figure 2)** (PEMSEA, 2019). And under fisheries, it was reported that the share of aquaculture in 2017 reached 56.4% of the total national fisheries GDP (Statistics Indonesia, 2018). This indicates that aquaculture can boost the national fisheries economic performance. From 2011-2015, aquaculture commodities showed significant increase. These commodities include seaweed (21.29%), shrimp (15.08%), fish (14.28%), and shellfish (9.94%) (**Table 1**) (PEMSEA, 2019).

The increase was due to several priority activities initiated by the government, namely: biofloc technology, rice-fish farming, feed self-sufficiency programs, and other technical assistance provided by DGA.

Based on the aquaculture production by commodity (**Table 1)**, marine culture production is higher than freshwater and brackishwater. However, the number is bigger for seaweed production rather than fish production. Moreover, it was reported that the total aquaculture production from marine aquaculture activities reached up to 9 million tons for seaweed commodities and the rest of 138 thousand tons for fish commodities in 2017. Thus, marine culture still must be developed further, given that the prospects appear positive. Indonesia still has great opportunities in developing marine culture. This can be seen from the existing potential area covering 12.1 million hectares while utilization is only about 285,527 hectares.

Therefore, the Indonesian government has been putting significant effort to develop marine culture production by improving the technology and enhancing the capacity for marine culture development. Indonesia has several advantages for mariculture development, including many potential mariculture sites, a stable tropical climate,

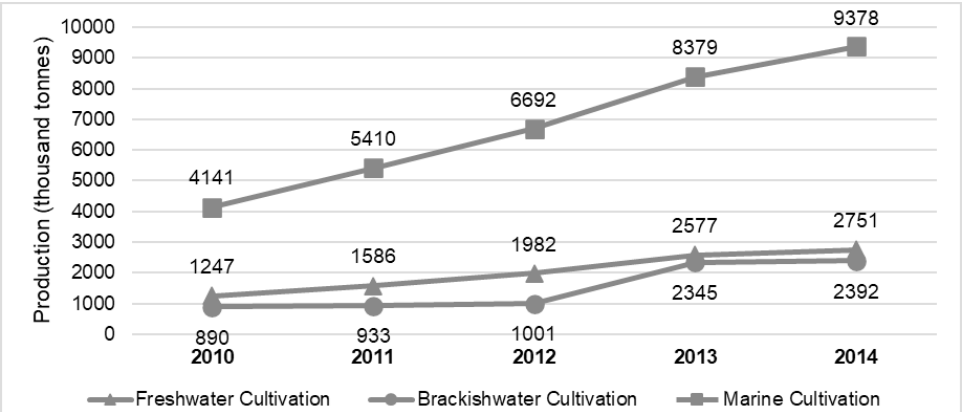

Figure 1. Trend of Aquaculture Production in Indonesia from 2010-2014 (PEMSEA, 2019)

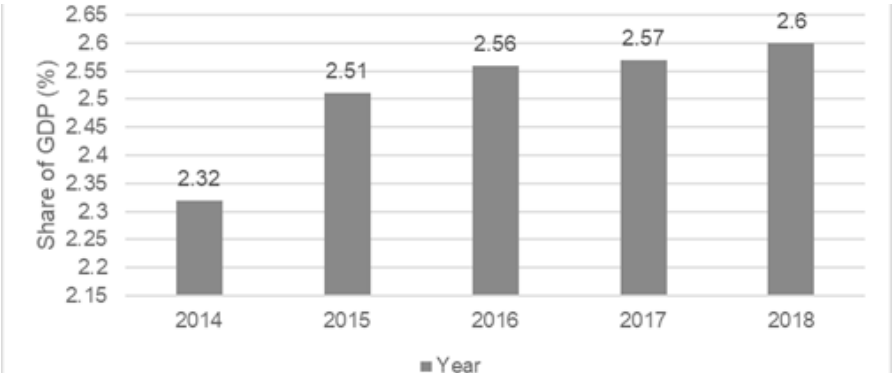

Figure 2. Contribution of Fisheries to the Gross Domestic Product (GDP) in Indonesia from 2014 to 2018 (PEMSEA, 2019)

Table 1. Aquaculture Production by Commodity from 2011-2015 (PEMSEA, 2019)

Commodity	2011	2012	2013	2014	2015	Growth 2011-2015 (% per year)
Seaweed	5,170,201	6,514,854	9,298,474	10,076,992	10,890,326	21.29
Shrimp	372,577	415,703	642,568	639,369	605,328	15.08
Black tiger	126,157	117,888	178,583	131,809	124,869	3.37
Vannamei	246,420	251,763	390,278	442,380	413,079	15.98
Other shrimp		46,052	73,707	65,180	67,381	17.29
Fish	2,068,472	2,550,255	3,116,988	3,390,080	3,486,917	14.28
Grouper	10,580	11,950	18,864	13,346	15,638	14.68
Seabass	5,236	6,198	6,735	5,447	5,123	0.49
Milkfish	467,449	518,939	627,333	631,125	668,262	9.60
Carp	332,206	374,366	412,703	434,653	461,882	8.63
Tilapia	567,078	695,063	914,778	999,695	1,068,604	17.59
Gourami	64,252	84,681	94,605	118,776	113,258	16.10
Pangasius	229,267	347,000	410,883	418,002	339,095	13.15
Catfish	337,577	441,217	543,774	679,379	722,657	21.31
Pompano			643	1,367	2,663	103.66
Silver barb	11,966	19,074	24,107	26,994	24,760	22.37
Bonylip barb	22,552	25,426	27,668	32,080	29,565	7.42
Giant snakehead	14,273	19,886	24,642	21,024	28,112	20.57
Shellfish	48,449	19,472	29,091	44,394	43,312	9.94
Others	269,264	175,269	213,785	208,295	200,351	-4.83
TOTAL	**7,928,963**	**9,675,553**	**13,300,906**	**14,359,129**	**15,226,234**	

and does not suffer from cyclonic storms. Globally, the demand for seafood products is expanding due to the increasing population and increased per capita consumption of fish products. Thus, the Indonesian government has been putting significant efforts to increase marine aquaculture production by developing the mariculture sector through the installation of off-shore cages in several locations in Indonesia.

Freshwater commodity such as African catfish (lele) is one of the most consumed and preferred fish by middle-class society in Indonesia. The African catfish dishes can easily be found everywhere, from a street food stall to a bigger restaurant. Apart from relatively more affordable, freshwater fish also tastes as good as seafoods. As a result, many fish farmers seize the business opportunity in freshwater aquaculture. For illustration, African catfish is one of the best-selling commodities in Indonesia. The cost of production and business analysis for extensive catfish culture using tarpaulin pool for small-scale/household business is provided below in **Table 2**.

With the assumption of up to 90% recovery rate, the initial 2000 catfish seeds stocked, harvest can reach up to 1800 pieces or about 500 kg. Given a market price of African catfish at about 10,000 Indonesian Rupiah (IDR)/kg (lowest price), harvest can yield up to 5,000,000 IDR. The price of African catfish range between 10,000 – 15,000 IDR per kg, depending on location and demand. Considering a production cost of 1,100,000 IDR, the net profit would be 3,900,000 IDR (Fatoni, 2016). This profit is for the first harvest. The next harvest can provide a bigger profit because pond installation cost has been deducted already from the first harvest. Prices also vary across cities, thereby affecting profit. However, this computation did not include labor cost and land rent. Also, note that this example used a tarpaulin pond with minimum capital. For businesses with bigger capital, a concrete pond can be built and used to co-culture African catfish with other freshwater commodities.

Table 2. The cost of production and business analysis for extensive catfish culture using tarpaulin pool (Fatoni, 2016)

Commodity	African catfish (IDR)
Initial capital	950,000
Tarpauline	600,000
Bamboo	300,000
Nails	10,000
Others	40,000
Production cost	1,100,000
Seeds (± 2000)	500,000
Feeds	500,000
Vaccine and Vitamin	100,000
Total	**2,050,000**

Issues and challenges

Indonesia still has to face several global challenges that require immediate action to solve the barriers to its sustainability. Issues and challenges encountered in the development of aquaculture can be identified and grouped into five big themes as follows:

Production system

a. Distribution of information and implementation of recommended technology have not been fully reached aquaculture business units in Indonesia due to the limited number of advisor (advance fish farmers) in the district. Technological guidance and assistance in mariculture areas have not been done intensively due to the remote location of certain areas.

b. Acceleration of Good Aquaculture Practices (GAqP/CBIB) certification is still an issue due to lack of socialization, assistance on GAqP application by facilitator/district service to fish farming units, fish farmers' lack of comprehension on GAqP standards and criteria, and that GAqP certificate is still done voluntarily and has no value-added for fish farmers who have applied the standard and criteria of GAqP, due to low acceptance of GAqP certificate in aquaculture processing units (UPI);

c. The application of GAqP in the aquaculture industry will also lead to the assurance of product safety, which does not exist yet in the industry, mainly for small-scale enterprises. Therefore, GAqP requires effort to solve the traceability issue of aquaculture products in Indonesia.

d. The price of registered fish feed is relatively expensive because most of the raw material are imported and are affected by dollar exchange rate and also influenced by "3F"- food, feed and fuel issues. Consequently, some fish farmers still use the relatively cheap unregistered fish feeds which are often of low quality.

Hatchery system

a. Limited information and distribution of high-quality broodstock and seed are among the issues in developing hatchery systems.

b. Low and inconsistent supply and poor quality of seeds. The development of seed supply systems is also constrained by the under utilization of facilities and infrastructure that have been built, mainly BBI/BBIP/BBUG (fish and shrimp hatchery) due to of lack of qualified human resources.

Infrastructure and facility system

The main constraint in the development of infrastructure system is limited infrastructure, such as water channel, both input/output that is damaged by siltation, difficult access to electricity network in aquaculture locations, difficult access road in aquaculture production location. In addition to infrastructure, limitations in aquaculture facilities have also become a constraint in the development of aquaculture systems.

Business system

One of the challenges in the development of a business system is capital, especially for the development of brackish water aquaculture or mariculture that requires substantial capital. Bank trust is still low towards fish farming. Bank requirements are quite difficult, thus access to capital for fish farming is also difficult. Another constraint is the institutional management system of POKDAKAN (fish farmers group) that has not developed well. Thus, weakening the bargaining position of fish farmers in terms of marketing.

Fish health and environment system

a. Diseases remain the main constraint in the development of a fish health system. Additionally, environmental degradation has also become an issue caused by uncontrolled pollution from other business sectors.

b. One of the most pressing environmental concerns on the development of aquaculture activity in Indonesia is the growing number of floating cages in lakes and reservoirs. The rapid development of floating cages activity has become more excessive and uncontrolled over the years. Thus, damaging the sustainability of the aquatic

environment that threatens the sustainability of floating cages. The problems are as follow:

i. Pollution and eutrophication from fish farming waste activities (fish droppings and leftover feeds). Pollution from human activities around the environment and upstream watershed.

ii. Frequent cases of mass mortalities farmed fish due to the up-welling phenomenon or the rise of the bottom layer of waters that contain many toxic substances to the surface.

iii. Excess carrying-capacity. Most cage culture activities have exceeded the environmental carrying capacity and caused some risk due to mortality and decreased production such as in Lake Toba (Maninjau) and Reservoir Cirata (Cirata, Saguling Darma).

iv. Excessive and uncontrolled cage activities spoil the tourism opportunities in the area.

Strategies

Product improvement through innovation technology

a. Aquaculture using biofloc system which is a technology for intensive culture based on the oxygen supply and specific floc microorganism.

b. Rice-fish farming to optimize use of field irrigation to increase production.

c. Fish feed self-sufficiency program where the fish farmers to are encouraged to produce their own feed using local raw material to save the cost of production. The government aids in the form of equipment and tools, together with expert advice.

d. The development of off-shore cage culture is one of MMAF's strategic programs to increase marine fish production. Target production from this sector reaches up to 2,448 tonnes per year of barramundi with an economic value of 13 million USD per year.

e. Recirculating aquaculture system (RAS) is an intensive fish farming system that uses infrastructure that allows the use of recirculated water. It applies filter physics, filter biology, UV, oxygen generator to control and stabilize the environmental condition, reduce the amount of water use, increase survival rates, and use of limited areas. With this RAS technology, the Freshwater Center in Tatelu, South Sulawesi was able to boost tilapia stocking rate up to 5,000 fish/m^3, while stocking density in conventional systems only reaches 50 fish/m^2. Productivity can be increased up to 100 times in the RAS system compared to the conventional system.

f. Seaweed tissue culture method or *in vitro* vegetative propagation technique to produce high-quality explants. The carrageenan content of seaweeds produced through tissue culture reaches up to 40% with an average LPH of 11.50%.

g. Fish resource preservation through fisheries management and restocking in open waters to increase fish population and preserve the diversity of fish resources.

Capacity building

Capacity building is one of the government's concerns to enhance human resources capacity both in knowledge and skills, particularly in aquaculture. The staff of the DGA and the fish farmers are subjected to capacity enhancement through trainings and workshops. The Ministry of Maritime Affairs and Fisheries (MMAF) works together with related institutions and partner countries in holding trainings and workshops on aquaculture.

Product improvement

The MMAF seeks to encourage the issue of traceability and food safety in aquaculture products to be addressed through process and product certification from upstream to downstream.

i. To ensure the availability of seeds, MMAF has established broodstock centers for the Jepara Brackish Aquaculture Aquaculture Center (BBPBAP) as a broodstock center and the tiger shrimp nauplii Center and merguensis, while the superior Shrimp Main Production Center and Shrimp (BPIU2K) Karangsem as vannamei shrimp broodstock center. Besides that, KKP is also trying to maintain the quality of shrimp broodstock through the issuance of Circular Letter (SE) prohibiting the use of parent from ponds.

ii. SNI development and aquaculture certification system (INDO GAP/ GAqP). The implementation of the GAqP system is very important as a guarantee that the aquaculture products produced are safe for consumption. GAqP is a series of considerations, procedures, and protocols designed to ensure that aquaculture activity is practiced in a controlled environment by taking into account sanitation, feed, medicine, biological materials, and chemicals (Kamaruddin *et al*., 2015). Currently, the Indonesian GAqP version has been harmonized with FAO guidelines on aquaculture certification, ASEAN shrimp GAP, and ASEAN GAqP guidelines.

Zoning

a. Environmental modeling and GIS-based tools for carrying capacity study to conduct site selection for appropriate aquaculture activity.

b. Cluster-based aquaculture which an integrated aquaculture management based on carrying capacity that is carried out collectively. A cluster approach is also considered as an effort to attaining group certification, thereby relieving small-scale farmers of the burden of bearing the high cost for this purpose (NACA, 2011). The benefit of implementing this system is to better control aquaculture management, such as a more stringent biosecurity system; better pond layout; more effective control of pest and fish diseases; better management of waste; and guaranteed traceability of production system.

Collaborative projects, government-initiated collaborative projects through several cooperation schemes, some of the recent collaborations are as follows:

i. FAO

a. Scaling-up of innovative rice-fish farming and climate change resilient Tilapia pond culture practices for blue growth in Asia (TCP/RAS/3603)

b. Supporting local fish feed self-sufficiency for inland aquaculture development in Indonesia (TCP/INS/3606)

c. Support mitigation of Antimicrobial Resistance (AMR) risk associated with aquaculture in Asia. (TCP/RAS/3702)

d. Traceability for shrimp farms (TCP/INS/3704)

ii. RI – Norwegia through Sustainable Marine Aquaculture Development in Indonesia (SMADI) project that works on 4 (four) themes: 1) marine spatial planning and carrying capacity; 2) disease and parasite control; 3) breeding and 4) technical standard for integrated off-shore aquaculture. This collaboration is implemented under the grant scheme provided by the Norwegian government. The purpose of this project is to assist the Indonesian government in sustainably developing the marine aquaculture sector by enhancing Indonesian administration and management systems.

Research and development

Some of the researches that have been done by the Research and Development Department under MMAF to support the DGA's program and activities are as follows:

i. Development of feed formula for increasing production and productivity of freshwater broodstock, 2015, Jojo Subagja, Mas Tri Djoko Sunarno, Research and Development Center for Freshwater Aquaculture, Sempur, Bogor.

ii. Pangasius culture (*Pangasionodon hypopthalmus*) based on local raw material feed, 2015, Ani Widiyati, Mas Tri Djoko Sunarno, Research and Development Center for Freshwater Aquaculture, Sempur, Bogor.

iii. Utilization of aquaculture recirculation system (RAS) for vannamei shrimp culture, 2013, Permana Ari Soejarwo, Center for Research and Engineering of Marine and Fisheries Technology, Indramayu, Jawa Tengah.

iv. *Gracilaria gigas* seaweed seed production technology with tissue culture method, 2015, Petrus Rani Pong Cook, Research and Development Workshop for Seaweed Culture, North Minahasa, Sulawesi.

v. Production of superior seeds of *Gracilaria* spp. seaweed through tissue culture, propagation in ponds and multi-location tests in the aquaculture center area, 2013, Rohama Daud, Sri Redjeki Hesti Mulyaningrum, Emma Suryati, Syarifuddin Tonnek, Research Center for Development of Brackish Water Aquaculture, Takalar, Sulawesi.

vi. Intensive production of superior fish in rice-fish farming land, 2012, Irin Iriana Kusnini, Otong Zenal Arifin, Vitas Atmadi Prakoso, Wahyulia Cahyanti, Freshwater Aquaculture Research and Development Center, Sempur, Bogor.

vii. Increased rice-fish farming productivity using organic fertilizers, 2012, Ani Widiyati, Yosmaniar, Adang

Saputra, Imam Taufik, Research Institute for Freshwater Aquaculture and Development, Sempur, Bogor.

viii. Intensification of Nila Best using biofloc technology, 2011, Yohanna Retnaning Widyastuti, Imam Taufik, Sutrisno, Research Institute for Freshwater Cultivation and Development, Sempur, Bogor.

ix. Application test of biofloc-forming heterotrophic bacteria in tilapia nursery, 2016, Eri Setiadi, Yohanna Retnaning Widyastuti, Ani Widiyati, Angela Mariana Lusiastuti, Research Center for Freshwater Aquaculture Fisheries and Fisheries Education, Sempur, Bogor.

However, more researches are still needed, particularly on marine aquaculture, to further study the feasibility of the marine aquaculture activity, the industry, and the impact of the activity on the environment.

References

Directorate General of Aquaculture (2015). *Strategic Plan 2015 – 2019*. Ministry of Marine Affairs and Fisheries, Republic of Indonesia.

FAO. (2017). FOCUS: fisheries and food security. *Sustainable Aquaculture Development*. http://www.fao.org/focus/e/fisheries/intro.htm. Food and Agriculture Organisation of the United Nations. Rome.

FAO. (2018). The state of world fisheries and aquaculture. *Aquaculture food fish production by region and selected major producers*. pp. 27. Food and Agriculture Organisation of the United Nations, Rome.

Fatoni, W. (2016). Pengangguran kaya raya. 1*01 bisnis rumahan yang melejitkan omzet*. Indonesia.

Investment Guideline for Sustainable Aquaculture in Indonesia 2018 - IDH - The Sustainable Trade Initiative. (2018). Retrieved from https://www.idhsustainabletrade.com/publication/investment-guideline-for-sustainable-aquaculture-in-indonesia-2018/

Kamaruddin, R., & Baharuddin, A. H. (2015). The importance of good aquaculture practices in improving fish farmer's income. *International Journal of Social Economics*, *42*(12), 1090-1105. doi:10.1108/ijse-02-2014-0028

Kementerian Kelautan dan Perikanan, Republik Indonesia. Statistik Perikanan Budidaya Indonesia. (2018). https://satudata.kkp.go.id/dashboard_produksi. Kementerian Kelautan dan Perikanan Budidaya. Direktorat Jenderal Perikanan Budidaya, Jakarta.

Network of Aquaculture Centres in Asia-Pacific. (2011). Better Management Practices (BMPs) and cluster management for empowering small scale farmers: scalling up strategies. National Workshop Report.

PEMSEA and Ministry of Environment and Forestry (Indonesia). (2019). National State of Oceans and Coasts 2018: Blue Economy Growth of Indonesia. Partnerships in Environmental Management for the Seas of East Asia (PEMSEA), Quezon City, Philippines. 440 p.

Rimmer, M. A., Sugama, K., Rakhmawati, D., Rofiq, R., & Habgood, R. H. (2013). A review and SWOT analysis of Aquaculture development in Indonesia. *Reviews in Aquaculture*, *5*(4), 255-279. doi:10.1111/raq.12017PEMSEA and Ministry of Environment and Forestry (Indonesia). (2019). National State of Oceans and Coasts 2018: Blue Economy Growth of Indonesia. Partnerships in Environmental Management for the Seas of East Asia (PEMSEA), Quezon City, Philippines. 440 p.

The World Bank Group. (2021). Aquaculture production (METRIC TONS) - Indonesia Retrieved from https://data.worldbank.org/indicator/ER.FSH.AQUA.MT?end=2018&locations=ID&start=1960&view=chart

Sustainable Aquaculture and Resource Enhancement in Lao PDR

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Introduction

Lao Peoples Democratic Republic (Lao PDR) is a landlocked country. Located in Southeast Asia, it is bordered by Viet Nam to the east, Cambodia to the south, Thailand to the west and south, and Myanmar and China to the north. It has rugged terrain, largely mountainous, but not in the developed part of the country. The surface area of Lao PDR covers 236,800 km^2. It suffers from limited infrastructure and a limited human capital base. It is home to many emerging social and economic institutions.

Fish and fisheries play an important economic role. Their contribution has been estimated at 7-13% of GDP by various surveys. The population depends heavily on the Mekong River and its tributaries, flood plains, swamps and rice fields for fish production. The waters are rich in biodiversity, but catches are declining due to increased fishing pressure brought on by increasing population and especially from modifications in the hydrology of the river due to implementation of irrigation, flood control and hydro-power development projects, deforestation and pollution.

Lao PDR has no outlet to the sea. It borders five countries. It is not a very populous country. Based upon the United Nation's estimates, its population is slightly more than 7.1 million people. Less than half of the population live in or around urban areas which are located near the rivers. Nevertheless, between 70 and 80% of the people in Laos PDR live a rural lifestyle and many of those living in rural communities rely heavily on aquatic resources for animal protein. Thus, fish and fish production are of critical importance.

There is substantial opportunity and resources available to enhance and augment aquaculture development in Lao PDR. It has abundant water resources and potentials for fisheries. Included in these are the Mekong River and its 14 tributaries, large reservoirs, shallow irrigation and weirs, wetlands and swamps, and rain fed rice fields. Lao PDR has twelve reservoirs which could possibly be used for fish stock enhancement practices. Other fish stocking sites have been identified. Almost 500 fish species have been found in Lao PDR including 22 exotic species and more are being identified (Vonglokham, 2017).

The fish farming systems are diverse and independent varying from rice farms, cages, ponds, community ponds and hatcheries. More than 18 indigenous species have been used in various types of fish farming: collecting the fry from the wild, artificial reproduction in captivity; and reproduction in fishpond culture by private and government hatcheries. Several common carp species which are easy to rear and are fast growing are being used for fish stocking.

Despite the steps which Lao PDR has

taken, significant challenges remain. To begin with, there is a need to gather baseline data for various types of water bodies, establishing simple and low-cost monitoring programs for selected water bodies and training the technical staff at all levels and obtaining the support and cooperation of local authorities.

It must be remembered, too, that Lao PDR is a poor country. Human and capital resources are in short supply and constant demand. In order to succeed, the focus must be on certain priority areas. Fish culture systems must be adapted to a specific area depending on local conditions. More focus must be given to discovering what works best and most productive in different fish farming systems. Finally, we must improve feeding techniques and the quality of the feeds.

Government estimates of fish consumption per capita in 2003 is 18 kg which accounts for 40 to 50% of animal protein intake (Mantingh, 2006). By 2020, the government has targeted fish consumption per capita at 20-23 kg.

Administration of fisheries and legal framework

In Lao PDR, fisheries and aquaculture activities are administered by the ministry responsible for agriculture. The Fisheries Section is lodged in the Technical Division of the Department of Livestock and Fisheries (DLF) of the Ministry of Agriculture and Forestry (MAF).

In 2007, the government undertook an overhaul of the major ministries which included the MAF. It was implemented by a committee which was established to improve the effectiveness of the administration by identifying institutional deficiencies and suggest re-organization of the ministry. The Fisheries Division was inaugurated as a division under the authority of the DLF. It enabled the fisheries and aquaculture issues to receive more prominence although it remains a small department with a small staff and limited financial resources. Subsequently there have been additional resources allocated so that fisheries and veterinary staff were strengthened in both the province and district levels.

It is worthwhile to draw attention to the National Fisheries Development Center (NFDC). It was the first facility in Lao PDR that housed both a training and research facility and which can organize international courses and workshops and hold international events and collaborate on research with foreign organizations. It has assisted in the promotion of target species such as common carp, tilapia and catfish.

Fisheries law

Beginning in the mid 1990's the government began to recognize the interconnectedness of aquatic resource management. There were series of laws enacted, i.e. The Agricultural Law, The Penal Law, Forestry Law, Water Resources Law and the Aquatic and Wild Animals Law. Those laws had been the basis for guidelines and instructions issued by local authorities to local communities for the management of aquatic resources as well as aquatic biodiversity of their areas. A new fisheries law was enacted in 2009. It is comprised of 10 chapters and 72 articles. The law defines the framework for implementing, managing, monitoring, and inspecting capture fisheries and aquaculture. It aims to promote aquaculture, conserve and protect fisheries resources for sustainable development and ensure the availability of fish and other aquatic animals for food security, contributing to the socio-economic development of the nation.

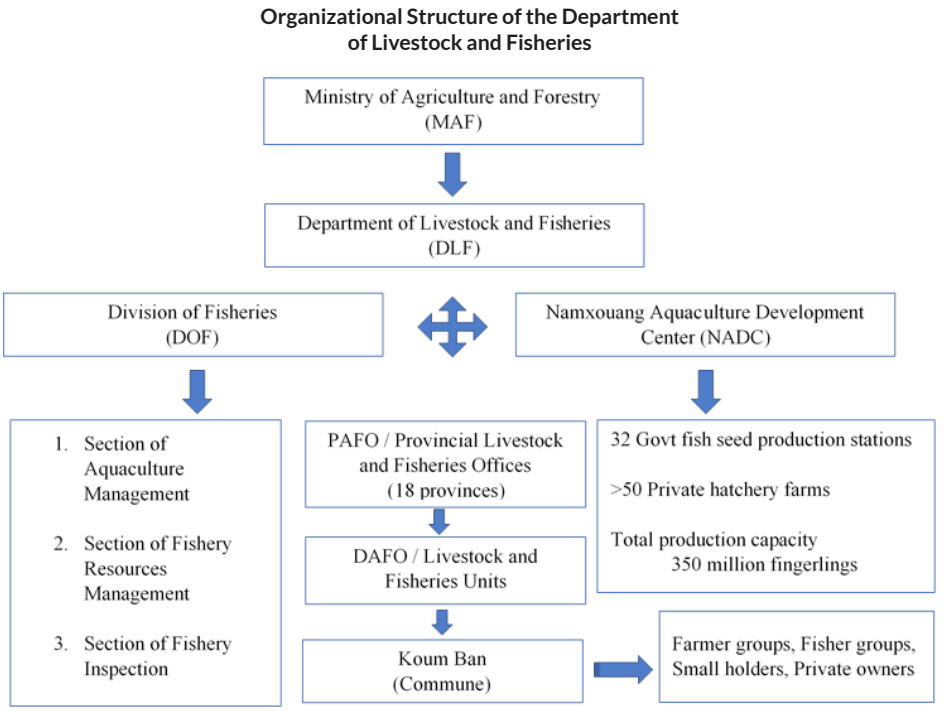

Figure 1. Organization Structure of the Department of Livestock and Fisheries (DLF) in Lao PDR (Phounsavath, 2015)

The law provides for community fisheries management and control measures indicating the right of local communities to manage and utilize their resources. In addition, the law empowers communities to establish village or community fisheries management committees for specific water bodies. The law also covers their organizational structure and roles and responsibilities in establishing fisheries protected areas and community ponds as well as the formulation of village fishing regulations.

The law provides for a very significant improvement of the former situation bringing fisheries management within one cohesive framework. The law provides for protection of aquatic resources and ecosystems and prohibits capture of designated threatened species and for some other species the law provides for special management by local authorities. Some articles prohibit destructive fishing methods such as, but not limited to, use of illegal fishing gear, explosives and electric current. Other articles protect breeding, nursing and feeding grounds including other ecosystems such as deep pools, fish conservation zones, important wetlands and rapids.

Water resources, fish, and fisheries in Lao PDR

Lao PDR can be divided into three physiographic zones: the Northern Highlands, the Annamite Range, and the Mekong Plain. The three zones are parts of four biogeographic units - the unit of Annam, the tropical lowlands, the tropical montane or mountainous country, and the sub-tropical transition zone. Approximately 88% of the total surface area of Lao PDR forms the catchment of the Lower Mekong Basin. The Mekong

and its 14 tributaries have been estimated to have an approximate water surface of 2,500 km^2. Additionally, there are many other streams and rivers draining to the Mekong River.

Capture fisheries and aquaculture are based on water resources ecosystems mainly consisting of rivers, streams, irrigation reservoirs, hydropower reservoirs, weirs, swamps, natural water bodies, flood plains, and wet season rice fields. The people of Lao PDR especially in the rural communities which account for nearly 75% of the population depend upon the country's fish and other aquatic animals as their most reliable sources of protein.

As mentioned earlier, nearly 500 fish species have been identified in Lao PDR which includes 22 exotic species. Other aquatic animals include about 37 amphibians, seven species of crabs and 10 species of shrimp. More than 18 indigenous fish species have been used in various types of farming and more than 20 species of freshwater fish are cultured in the country. Some of those species are Chinese carp, grass carp, bighead carp, tilapias, African catfish snake head and silver barb. Fish culture can reward the farmers financially as well as nutritionally.

There are several fish farming systems common in Lao PDR: pond fish culture, integrated fish-livestock farming, rice-fish farming, cage fish culture, and culture-based capture fisheries.

Studies conducted in neighboring countries have shown the viability of integrating agriculture, aquaculture and livestock farming as waste from one system can be recycled in the other system. This fish farming system is only applicable in rural households. Most households are small farm holders who grow rice and raise some livestock such as pigs, chicken and ducks. Integrating different farming systems can assist small farm holders who face obstacles in obtaining and paying for feeds. Although this system may help small farm holders it will have minimal impact on the overall goal of increasing fish production.

Rice-fish farming has been practiced for centuries. The government has sought to codify provisions governing access to rice field fisheries. Initially, it recognized two separate regimes of access depending on whether the area where the rice fields are located is flooded. Normally, a person wishing to fish in a rice field must obtain permission from the field owner or from whoever has the land use right over that field. However, in the event of a flood there is no requirement to obtain permission. Farmers in the north objected to this provision since their rice fields are flooded for a much shorter time than in the south and those farmers feared damage to their rice crops. The laws were adapted to reflect the diversity of practices throughout the country. Nevertheless, rice-fish farming like integrated fish-livestock farming has little impact on the overall goal of increasing fish production.

Unlike rice-fish farming and integrated fish-livestock farming, fish cage culture has the potential to significantly impact fish production. Farmers who do not have access to ponds can practice aquaculture in bodies of surface water such as reservoirs, rivers, irrigation canals, etc. In neighboring countries, farmers grow high value species with supplemental feeding. Fish culture in cage should encouraged as well as regulated.

In many villages in the country, there are communal ponds and water bodies used by village inhabitants as open access for

fishing. Some villagers stock fingerlings of various species to supplement the wild fish in the water body. This system of culture-based capture fisheries can serve to augment the local diet and source of nutrition to the villagers. Where possible, this practice should be encouraged and aided by government resources.

National strategy for fisheries management and development

It is the government's highest priority to obtain food self-sufficiency in agriculture and fish products. This is expected to aid in overcoming poverty in rural areas, improve the nutritional level and economic status. Current national agricultural and fisheries development policies will center around the following objectives (USAID Oceans and Fisheries Partnership, 2020):

1. To meet food security, especially protein intake. It is estimated that 33% of Lao PDR children under 5 years of age PDR suffer from stunting i.e. impaired growth and development due to poor nutrition;

2. To ensure the provision of fishery products as commercial commodities for local markets and for future export;

3. To support the rural development to alleviate poverty and create income generating opportunities;

4. To reduce slash and burn cultivation by integrating fish culture into the upland farming systems;

5. To contribute to the sustainable use, appropriate management and protection of aquatic resources including aquatic biodiversity.

6. To upgrade and establish basic infrastructure required for further aquatic resources research management and development.

7. To strengthen, upgrade, and perform the technical support services in research, extension, management, and development of subsectors such as Living Aquatic Resources Research Institute, Inland Fisheries Development Center, and AquatiAnimal Health Diagnostic Network.

DLF Policy for aquaculture development focus on priority areas.

In conjunction with the National Strategy the DLF has adopted the following strategies to focus on the following priority areas:

1. Assess traditional fish culture systems and flood plain resource potentials in different agro-ecological zones.

2. Search for and promote appropriate types of extensive and semi-extensive farming systems.

3. Search for approaches and efficient interventions or extending fish seed distribution networks.

4. Search for and develop appropriate extension approaches for upgrading the many small holder farmers in rural areas.

5. Fish disease prevention.

6. Improvement of feeding techniques and quality of feeds.

Challenges

Lao PDR is a small country with a small population. Although the economy of Lao PDR is one of the world's fastest growing, it is still one of the poorest countries in the region. It has a large but uneducated workforce. A large portion of its economy is maintained by foreign investment. Lao PDR has a great deal of hilly and mountainous national land with different climate areas. Although it has abundant traditional fish varieties, it makes poor use of improved varieties. It has the potential to harness organic aquaculture, but currently makes limited use of inputs.

Recommendations

The development of aquatic resources should be given a higher priority by the government since it is a key component in improving food security. Aquatic resources have the potential to generate cash income, provide employment opportunities, and diversify aquaculture as well as agricultural development.

Lao PDR should monitor capture fisheries as regards to resource management, as well as promote sustainable aquaculture. Research and development issues mandate balance development between aquaculture, fisheries and the aquatic environment. Each sub-sector demands technical development, training at all levels, and the involvement of higher education.

As mentioned earlier, there is a need to gather baseline data for various types of water bodies, establishing simple and low-cost monitoring programs for selected water bodies. It is also necessary to conduct surveys and research on the protection of fisheries production and biodiversity, while keeping in mind the habitat change, overfishing, pollution and the impact of newly introduced species.

Flexibility is critical. Farmers have been culturing fish without government inputs. New methods must be introduced with care and the farmers must be convinced of the benefits of change. Farmers and villages must be encouraged to consider and implement fish culture in cages and the DLF must be ready to assist in the implementation.

References

Mantingh, I. 2006. The Lao People's Democratic Republic. Retrieved from http://www.fao.org/fi/oldsite/FCP/en/LAO/profile.htm

Phonvisay, S. 2013. An Introduction to the Fisheries of Lao PDR. Mekong Development Series No. 6, 62 pages. Mekong River Commission, Phnom Penh, Cambodia. ISSN 1680-4023

Phounsavath, S. 2015. Promoting co-management in inland fisheries: Experience of Lao PDR. *Fish for the People*, *13*(3), 33-40.

USAID Oceans and Fisheries Partnership. 2020. Catch Documentation and Traceability. Retrieved from https://www.seafdec-oceanspartnership.org/resource/usaid-oceans-workshop-report-on-the-ecdt-showcase-for-the-mekong-region/

Vonglokham, K. 2017. Fisheries country Profile: Lao PDR. Retrieved from http://www.seafdec.org/fisheries-country-profile-lao-pdr/

Promotion of Sustainable Aquaculture in Malaysia

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Abstract

Aquaculture has been identified as a priority sector in the development of Malaysia's economy. It receives a wide participation as a result of the progressive development in most parts of the country. From producing only about 7% of the national fish production in 1992, aquaculture has produced almost 13% to that of capture production in 2003, and expected to produce equal volume to the latter in the future. The aquaculture production leaped from less than 80,000 metric tonnes in 1992 to more than 427,000 metric tons in 2017 valued at MYR3 billion. Demand is expected to continue to grow with anticipated population growth. Aquaculture provides employment, business and investment opportunities in this country. As of 2017, there are over 18,000 aquafarmers in Malaysia, with a total farm size of more than 34,000 ha. Two key factors i.e. the physical and financial factors, have boosted the competitiveness of Malaysia's aquaculture industry. The National Key Economics Area (NKEA) has become a mechanism to allow big players to lead private sectors participate in this industry. This paper intends to explicate Malaysia's aquaculture potentials with a view to provide insight prospects for aquaculture growth.

Keywords: aquaculture, sustainable, fisheries, Malaysia

Introduction

World population is expanding from 7 billion in 2011 and estimated to reach 9 billion in 2050 (Teh, 2012). Therefore, growth in human population also means increase in food intake. Fish and fish products have become essential diet components for most of the world's population. Currently, world fish supply is predicted to increase to 187 million metric tons (mt) by 2030. Nonetheless, capture fisheries is observed to remain stagnant over the period of 2000-2030 and global aquaculture projection will progressively increase until it reaches the points where it equals production from captured fisheries by 2030 (World Bank, 2013).

Global aquaculture continues to grow faster than other major food production, contributed nearly 47% of the world's fish, with an average annual growth of 5.8% during the period 2000–2016 (FAO, 2018). Global farm food fish production was 32.4 million mt in 2000 up to 55.7 million mt in 2009 and 80 million mt tons in 2016 (Roslina and Amir, 2014; FAO, 2018). Asia-Pacific is the highest world aquaculture producer with 92.5%. The largest quantities are from China accounting for more than a third of global production (World Bank, 2013). The world population is estimated to reach 8 billion and 2.2 million metric tons of fish need to be produced to meet the demand for an annual per capita consumption of 29 kg (Rosita, 2017).

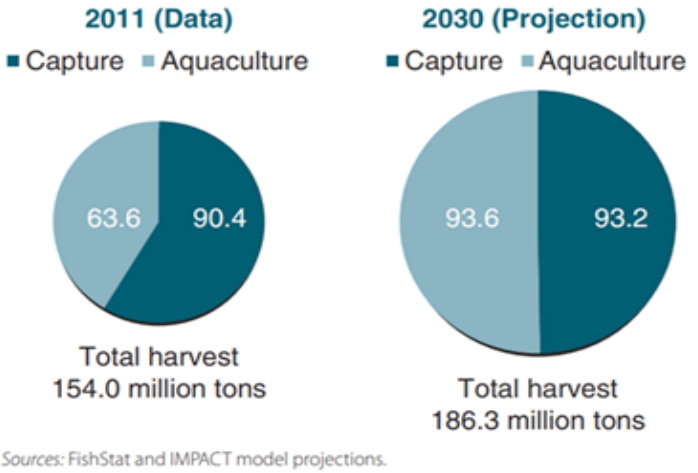

Figure 1. Aquaculture vs. capture fisheries from 2011 to 2030

Fish and fisheries products are popular sources of protein in Malaysia. The demand for fish is expected to increase from 1.3 million mt in 2010 to 1.9 million mt by 2020 (DOF, 2015b). In 2017, the total fishery production of the country has reached 1.93 million mt with a total value of MYR14.1 billion (DOF, 2019). Fisheries sector is composed of two major components, namely marine capture fisheries and aquaculture. They make vital contribution to Malaysia's food security and provide important livelihood opportunities and income for many subsistence fishing and farming families (Fisheries Research Institute [FRI], 2017a). Fish is presently the most inexpensive source of animal protein consumed by the commoners and it is a typical menu for Malaysians. According to the Food and Agriculture Organisation (FAO) Malaysia is among the top fish consuming nations in Asia (54.5 kg/year in 2010). The value goes higher in 2014 at 56.5 kg per capita higher than Thailand and China, but still below the levels in Japan and South Korea (Teh, 2012).

Current scenario of aquaculture industry in Malaysia

Malaysia's aquaculture began in the 1920s, starting with the polyculture of Chinese carps in ex-mining pools, followed by shrimp farming using trapping ponds in the middle of 1930s, and then culture of blood cockles in mud flats in early 1940s (Teh, 2012; Rosita, 2017). Cage aquaculture started in the early 1970s with the semi-intensive culture of shrimp, and the sector has significantly expanded in recent years (Kechik, 1995). Presently, Malaysia's aquaculture includes marine, brackish water, and freshwater aquaculture.

The aquaculture industry in Malaysia is still small compared to its neighbouring countries such as Thailand, Indonesia, Philippines and Viet Nam (Roslina and Amir, 2014). However, Malaysia also supplies aquaculture products to other countries through exports. Malaysia's major export products are freshwater fish

(tilapia and catfish) shrimps (*Penaeus* spp.), marine finfish (groupers, snappers and seabass), molluscs (cockles, mussels, and oysters) and seaweed (**Figure 2**).

Since 2003, the Government of Malaysia has implemented many programs to boost the potential of this industry. The government has committed an enormous allocation of physical and financial services to various aquaculture projects, particularly Aquaculture Industrial Zone (AIZ) projects. Basically AIZ are designated zones for both lands and water bodies which are granted by the state government for commercial scale aquaculture projects. The establishment of AIZ has transformed the aquaculture sector into a more technological activity driven by high market contribution in order to increase national food production and resolve the shortfall in captured fish production. In that particular year, aquaculture production reached 196,874 mt valued at over MYR10 billion, and contributed about 13% of the total fisheries production. Brackishwater aquaculture, with a total volume of 144,189 mt and covering an area of 17,357 ha, represented almost 70% of aquaculture production in terms of both quantity and value (DOF, 2003).

Since then, aquaculture has been acknowledged as a strategic industry that can accomplish the local demand for high value protein resources as well as demand for fish products for trade purposes. This has facilitated the government to reach its goals in the Ninth Malaysia Plan for food production growth of 33.4% or 1.8 million mt for fisheries and reached 103% self-sufficiency level by 2010 (Teh, 2012). The aquaculture industry benefited the country at both domestic and international levels by reaching the target for fish production and also recognized private sector technical and research capabilities for economic growth (Rosita, 2017).

The importance of aquaculture production in Malaysia's economic growth continued to be highlighted in the National Agro-Food Policy (NAFP 2011-2020) as the main focus in boosting the competitiveness of Malaysia's agriculture sector. The NAFP, which was launched in 2011, is the current policy by the Malaysian government in agriculture. Overall, the centre stage in NAFP is maximizing aquaculture contribution to food security and to national income and export earnings as well as maximizing income of aquaculture producers (Othman *et al*., 2017).

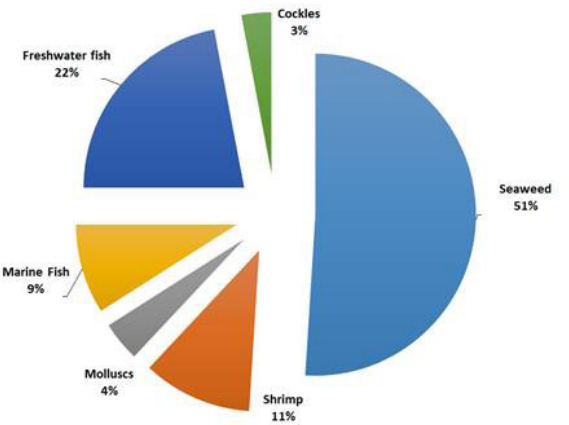

Figure 2. Aquaculture production in Malaysia in 2015 (Othman *et al*., 2017)

Earlier during the Third National Agricultural Policy (NAP3 1998-2010), a prospective development plan for aquaculture beyond 2010 was projected with a production target of 508,000 mt annually. Under NAFP, the target was further pushed to 790,000 mt, with an annual projection growth of 8.6% (Baki *et al*., 2012), as shown in **Figure 3**.

The aquaculture sector is recognized as one of the important drivers of economic activities under the Malaysia National Key Economics Area (NKEA). Sixteen agro-food Entry Point Projects (EPP) of the NKEA are listed under aquaculture. The government has allocated MYR18.9 billion for the 16 EPPs, where 70% sharing are from private sector (local and foreign) and the balance are supported by the government. These projects receive prioritized government support and focuses on transforming a traditional small-scale, production based sector into large scale business industry. The EPPs and Business Opportunities (BO) are driving the growth of the private sector while allowing them to get involved and offer opportunities for investment. Under BOs, the related program helps to create opportunities for aquaculture in terms of future growth such as branding and marketing. On the other hand, the EPP program aims to establish growth in food production. Here, three sectors for aquaculture have been identified namely ornamental fish farming, feed mills and export centres.

Currently, under the EPPs, DoFM has engaged with commercial-scale seaweed farming in the east coast of Sabah, integrated shrimp aquaculture zones and integrated cage culture with different goals. The aim of seaweed farming is to create high-yield commercial scale business by clustering farms under mini-estate initiative. The production of seaweed is projected to rise from 20,000 tons dry weight in 2010 to 150,000 tons dry weight by 2020 (Othman *et al*., 2017). Meanwhile, the EPP on shrimp sector seeks to increase production of fully export premium quality shrimp to all major markets. This can only be achieved by zoning the affected areas in integrated way and fully equipped with infrastructures and facilities such as hatcheries, processing plant, feed mill, grow-out pond areas and driven by anchor company. Target for cage farming is to increase production of high-value commercial fish species such as seabass, groupers, snappers and tilapia. It is projected that the fish production from this project will increase to 28% of the

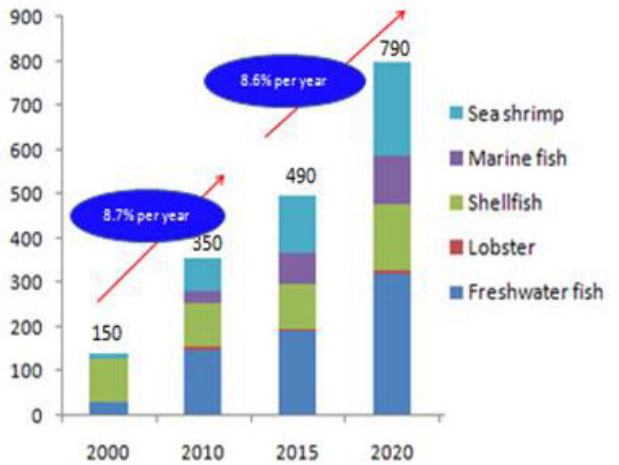

Figure 3. Target aquaculture production 2000-2020 ('000 mt)

total volume of the aquaculture production (Othman *et al*., 2017).

In 1992, this sector employed nearly 18,000 people who were mostly involved in freshwater farming. The total pond area was 8,360 ha and a total of 426,846 m^2 of cage were used (Kechik, 1995). Since then these numbers have grown rapidly. In 2013, there were about 1,966 brackishwater cages and 84 brackishwater pond entrepreneurs located in Malaysia. The total land used for the brackishwater aquaculture projects was 2,861,069 m^2 for cages and 6,903 ha for ponds (Rosita, 2017).

In 2017, aquaculture production in Malaysia has contributed food-fish of 427 millions mt valued at MYR3.041 billion (**Figure 4**). There were 18,378 aquafarmers in Malaysia, mainly in the freshwater sector, with the total farm size of 34,300 ha (DOF, 2017). In terms of percentage share of gross domestic product (GDP) in the national agriculture sector, the aquaculture sector consistently contributed significantly from 6.7% in 2010 to 8.9% in 2016 (DOS, 2016).

The aquaculture industries do have some issues and challenges. Among the common issues faced by these industries are lack of local workforce. Malaysia's aquaculture industry depends on 7,850 registered foreign workers (Othman *et al*., 2017). Other than that, small-scale farmers in Malaysia are burdened with high cost of farm operations, especially since 60-70% of cost are attributed to commercial fish feeds. In addition, lack of good quality of fry and broodstocks, emerging of new fish diseases such as Tilapia Lake Virus (TiLV) and Early Mortality Syndrome (EMS) / Enterocytozoan Hepatopenaei (EHP), and export barriers imposed by developed countries such as America gives a great pressure and challenge to farmers. The issue on quality and safety of fisheries products varies from one importing country to another and it changes from time to time. In facing these issues, the local entrepreneurs need to be more sensitive and further explore new markets to ensure that the excessive volume of fisheries products in domestic market could be exported profitably. At the same time, the products need to be produced in a cost-effective manner, realizing that the local production cost is getting higher compared to top competitors such as China, Indonesia, Viet Nam and Thailand. These issues and challenges, however, could be managed successfully with the effective communication and up to date information (Sharihan *et al*., 2018).

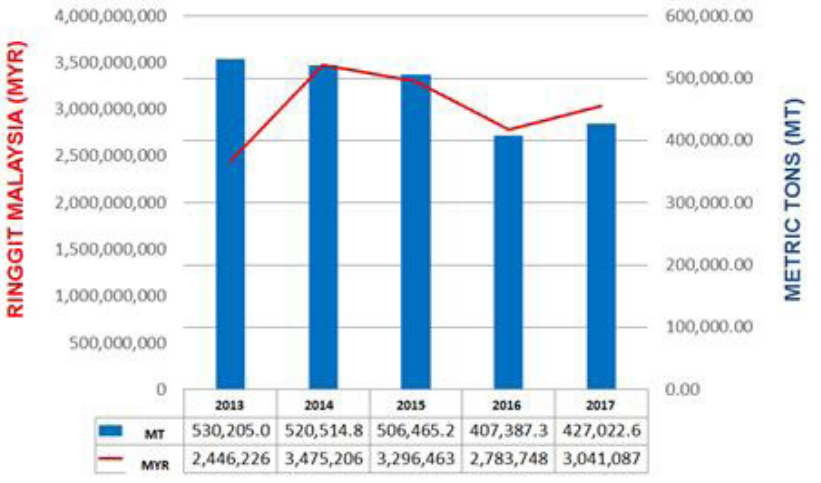

Figure 4. Malaysia aquaculture production (2013-2017)

Way forward: Aquaculture as a new frontier in food production in Malaysia

The Department of Fisheries Malaysia (DoFM has taken serious effort to achieve the Key Performance Indicator (KPI) for aquaculture sectors. To initiate the overall production, three sectors of aquaculture implementations were identified. They are (i) NKEA aquaculture project implementation and set to produce 33% of the required volume; (ii) development of new aquaculture areas which will produce 27% of the production; and (iii) increase productivity from the current operation hence to produce 40% of the total fish production (**Figure 5**).

Since aquaculture has been recognized by the Malaysian government as one of the priority under the NKEA focus, it suits as an alternative to improve the living standards of rural population. The NKEA has become a mechanism to allow big players to lead private sectors participate in this industry, helping to enhance national economic activities by creating many job opportunities, producing high value fish and fish products, and creating aquaculture chain productions from hatcheries to marketing level. In total, NKEA projects have created jobs for 2,872 people, invested approximately MYR410 million in 36 EPP. In 2018, about 23,602.74 mt of fish and fishery products were recorded with sales of MYR660.87 million (DOF, 2019).

Malaysia is the first country in Asia to embrace and implement Good Aquaculture Practice. It was launched as myGAP on 28 August 2013. myGAP is a comprehensive certification scheme for agricultural, aquaculture and livestock sectors. DoFM has produced documentation and guidelines on Good Aquaculture Practice – Aquaculture Farm (GAqP) for each aquaculture system. It is a very inclusive guideline that covers all the critical criteria of food safety and environmental concerns. GAqP is a scheme for aquaculture farm while General Guidelines and MS 2467:2012 Code of Practice used for seaweed cultivation. Compliance of myGAP will not only benefit farmers to produce fresh and best quality fish but the most important aspect is customers trust and rights to have safe and reliable food to consume. In addition, it will also increase Malaysia agricultural products competitiveness at the international level. There are still more rooms for MyGAP direction to achieve such as increase number of farms with MyGAP certification to encourage sufficient production of quality and safe agricultural products for domestic consumption and international markets, increase customer's awareness and demand for quality and safe agricultural products with MyGAP logo, and benchmark with international GAP certification scheme such as the ASEAN GAP and Global GAP. To date, about 70% of farmers in Malaysia has been certified with MyGAP that make them eligible to export their products to Singapore, Korea, Australia, America and European

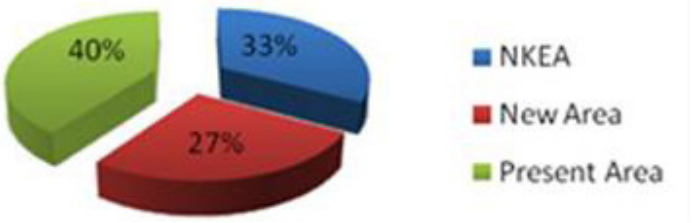

Figure 5. Source for aquaculture production

countries. At least 395 farms are expected to be MyGAP certified by 2020 (DOF, 2019).

DoFM has introduced a new concept of synergy farming in dedicated aquaculture zones called Aquaculture Industrial Zones (AIZ). In 2015, there were 56 AIZ sites with a total area of 24,388 ha (DOF, 2016). The overall projected AIZ production is 400,000 mt with a value of MYR5.8 billion, focusing on highly commercial species, such as tilapia, groupers, penaeid shrimps, mussels and seaweed (Baki *et al*., 2012).

One of the successful synergy farming is located in Tasik Temenggor, Perak, covering a water body of approximately 100 ha. The new strain of Genetically Improved Farmed Tilapia (GIFT) was used in this farm. The marketing chains are mainly for overseas such as America, Middle East countries and Singapore. As for mollusc culture, Malaysia has traditionally cultured green mussel (*Perna canaliculus*) using the longline method and it represents the second most important bivalve species after the blood cockle (*Tegillarca granosa*). Nowadays, Malaysia is using a new technology called mussel smart line farming that can produce approximately 10 mt per line with minimal workforce. This system has been introduced to 25 participants in Johor, and has generated income to this community up to MYR3.6 million (DOF, 2019).

In addition, a comprehensive plan named Aquaculture Project Implementation (API) with adoption of quick-win and high outcomes to drive viable and sustainable aquaculture activities has been implemented. Some of the strategies proposed in the API include upgrading existing entrepreneurs' projects and having engagement sessions with local agencies such as Muda Agricultural Development Authority (MADA) and Kemubu Agricultural Development Authority (KADA) to provide general release programs or restocking for species suitable for canals, paddy fields, mine sites or lakes. Private sectors interested in undertaking the program will be identified and assistance provided (MOA, 2019).

Cluster approach for several existing projects in the states has been implemented by focusing on one species such as the freshwater prawn project in Negeri Sembilan, the fish cage farming project in Pahang and the cockle project in Johor. Special teams have been established as management and technical advisors that comprise representatives from the Fisheries Research Institute (FRI), Aquaculture Development Center and the State Fisheries Office.

It is undeniable that technology and innovation had play an important role to aquaculture production in the world, as well as in Malaysia. DoFM has furnished the strategic plan and the key elements within the plan of action in order to enhance the productivity in aquaculture industry through research and development, technology and innovation. Some of the new technologies and innovation that have been introduced in aquaculture sectors are the production of Red Tilapia, blue Kelah fish and Recirculating Aquaculture System.

Tilapia has always been the favourite fish among locals in Malaysia. With red colour characteristic similar to marine fish, moderate market price, easy to breed and rear, it makes Red Tilapia one of the major species for aquaculture. Currently, tilapia hatchery operators have just bred fingerlings in mass production without having proper information on their origins, genetic background, growth performance and pedigree information and then sell directly to fish farmers. However, the aquaculture sector is facing lack of high-quality seedstock for production. Therefore, DoFM has taken initiative to

produce Red Tilapia with faster growth and resistance to diseases through genetic improvement program using selective breeding method (FRI, 2017b). The program has successfully developed selected strains that produced higher growth rate (2.8 g/day) and higher survival rates (78 %) compared to unimproved strain of Red Tilapia (Azhar *et al*., 2017). Thus, the improved strains are valuable genetic resources for aquaculture industry. Moreover, it could reduce the operational cost.

Kelah fish (*Tor* sp.) is one of the most expensive fish in Malaysia where its price can fetch up to MYR1000/kg. According to DOF (2015a), the production of Kelah in 2015 was 24.76 mt and it dropped to 19 mt in the following year due to difficulties in obtaining seeds. Most hatchery operators collected Kelah seeds from its natural habitats. This method contradicts with the general practice and regulation of natural fisheries resource conservation. In this regard, the DoFM has implemented the development program for Kelah to produce potential and competitive new varieties that can compete with existing aquaculture species in Malaysia (FRI, 2017b). Among the benefits of the program are as follows:

- Increase the hatching rate from 50 % to 90% via a simple nursery system.
- Increase the incubation rate of eggs from one to four times a year through the provision of a formulated diet specifically for the matured bloodstocks.
- Improved the quality and quantity of eggs produced by each broodstock.

DoFM has successfully introduced an innovation called the Cheap Efficient Nursey Tank System (CENTS) which can produce more efficient and cheaper tank system to address problems of seedling. The system used continuous flow of water that connected the nursing tank to the pump from the improved water storage tank with the Recirculating Aquaculture System (RAS), making the CENT-RAS system a unique innovation by DoFM. The system offers cheaper, more efficient, economical, minimal time management and flexible features, and enables the salinity to be adjusted to the level required in order to minimize the usage of seawater. This system has resulted in higher production of marine fish by up to 90 % from 20 kg/tons to 64.4 kg/tons.

In terms of fish health management, DoFM has developed SirehMax, an innovation product from FRI, that could inhibit the growth of microbial culture (FRI, 2017b). It is capable of replacing antibiotics by increasing the marine life expectancy by more than 80 % with a much lower treatment cost of MYR0.40/kg food per treatment compared to MYR1.40 for antibiotic costs. SirehMax is not only effective in controlling marine fish diseases but is also designed to be user friendly and environmentally friendly.

In addition, DoFM has produced grouper hybrid via its spawning technology which has managed to increase the cycle of farming rate by reducing the breeding period from a year to just 10 months with a high survival rate of 76 %. This technology has also reduced its predatory features compared to tiger grouper. The sale price of this hybrid grouper eggs is around MYR6,000 to MYR10,000 per million eggs. While the price of 10–12 cm juveniles is between MYR5–MYR7 per individual.

The potentials of aquaculture can further be developed through the advancement in biotechnology as a means to enhance productivity via improved yield and quality of production. Potential benefits

include improving growth rate and cost effectiveness; increasing resistance to environment and pathogens; improving broodstock quality and control reproduction; and creating new and better products. The main challenges in the application of biotechnology in Malaysia are quoted as limited financial resources, lack of qualified personnel, less optimal structure for cutting edge research, and limited international collaboration.

Overall, aquaculture development in Malaysia continues to sustain due to its strategic location in the heart of Southeast of Asia. Blessed with abundant fisheries resources, good climate and generally safe or free from natural disasters, Malaysia offers the perfect ambience for aquaculture to grow steadily in the future and provide ample supply of raw materials for a wide range of seafood industries. With strong support from the government of Malaysia under the NAFP (2011-2020) and National Economic Transformation Programme as part of the government's strategy, aquaculture supply will continue to increase and will help to improve the balance of trade and expand Malaysia's export.

Conclusion

Aquaculture in Malaysia is the forefront of fish production for national food security. However, the growth rate of development is not significant. Good and efficient aquaculture practices in farm management will influence the potential growth of this sector and minimize farm production risks and vulnerability. At the farm level, all aspects of aquaculture farm management have direct bearing on the sustainable growth of this sector currently and in the future. While at the same time, ensure that aquaculture practices remain sustainable and safe. This will produce high quality aquaculture production including effective land use management as well as technical factors such as labour, feed management, harvesting, and marketing the products. Undeniably, technological assistance will help enhance the future growth of aquaculture. Thus, the creation and availability of low-cost and efficient aquaculture technology may help aquafarmers in enhancing their production. While big enterprises fund their own research for technology improvement, this is not applicable to more than 80% fish farmers in the country, which are small scale and family farm operators. Technology and innovation must be initiated and sourced by the government in order to improve the aquaculture sector. It is the objective of DoFM to manage the fisheries and aquaculture into an economic, profitable and sustainable industry in the long run and at the same time to protect and rehabilitate the marine habitats and ecosystems.

References

Azhar, H., Ngo, P.T., and Nguyen, H.N. 2017. Genetic analysis of a red tilapia (Oreochromis spp.) population undergoing three generations of selection for increase body weight at harvest. J. Appl. Genetics. DOI 10.1007/s13353-017-0411-8.

Baki, B., Azirah, H., Che Wan Jasimah, M.R. and Songan, P. 2012. The new Malaysian National Agro-Food Policy: Food security and food safety issues. Paper presented at the 3rd. International Conference on Global Environmental Change and Food Security (GECS-2012). Marrakesh, Morocco. November 2012.

Department of Fisheries (DOF). 2019. Kajian semula pelan strategik Jabatan Perikanan Malaysia 2019-2020. Department of Fisheries, Malaysia.

DOF. 2017. Annual Fisheries Statistic. Vol.1. Department of Fisheries, Malaysia.

DOF. 2016. Annual Report 2015. Department of Fisheries, Malaysia.

DOF. 2015a. Annual Fisheries Statistic. Department of Fisheries, Malaysia.

DOF. 2015b. National plan of action for the management of fishing capacity in Malaysia. (Plan 2). Department of Fisheries, Malaysia.

DOF. 2003. Annual Fisheries Statistic. Department of Fisheries, Malaysia.

Department of Statistics (DOS). 2016. Selected agricultural indicators, Malaysia, 2016. Department of Statistics, Malaysia.

Fisheries Research Institute (FRI). 2017a. The state of the aquatic resources. Department of Fisheries, Malaysia.

FRI. 2017b. Annual Report 2017. Fisheries Research Institute, Malaysia.

Food and Agriculture Organisation (FAO). 2018. The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

FAO. 2012. The state of world fisheries and aquaculture. 2012. Food and Agriculture Organization of the United Nations, Rome.

Kechik, I.A. 1995. Aquaculture in Malaysia, pp. 125-135. In: Bagarinao TU, Flores EEC (eds) Towards sustainable aquaculture in Southeast Asia and Japan. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Ministry of Agricultural and Agro-based Industry (MOA). 2019. Prioriti dan strategi 2019-2020. MOA, Putrajaya.

Othman, M.F, Mazuki, H., Yeo, M.E, Amal, M.N.A., Natrah, I., Ho, G.C and Merican, Z. 2017. Transforming the aquaculture industry in Malaysia. World Aquaculture. Vol. 48(2): 16-23.

Rosita, H. 2017. Climate change and the aquaculture sector of Sarawak (Doctoral dissertation). University of Malaya, Kuala Lumpur.

Roslina, K. and Amir, H.B. 2014. The importance of good aquaculture practise in improving fish farmer's income. International Journal of Social Economics, Vol. 42(12): 1090-1105.

Sharihan, F., Aizul, N.H., Shuib, R. and Noor Azira, T. 2018. Current issues in aquaculture: Lessons from Malaysia. Advanced Science Letters, 24(1): 503-505.

Teh, E. 2012. Fisheries in Malaysia: Can resources match demand? Sea Views. No.12/2012.

World Bank, 2012: Fish to 2030: Prospects for fisheries and aquaculture. Agriculture and environmental services discussion paper, no.3. Washington DC.

Sustainable Aquaculture and Resource Enhancement in Myanmar

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Abstract

Myanmar is located in a rising global fish demand zone for both processing and fish consumption, and has relatively rich land, water and coastal resources, as well as a diversity of agro-climatic conditions. Myanmar has taken its first major step towards developing a sustainable aquaculture industry that will meet domestic nutritional needs and support the fishery export business at a time when wild fish stocks are declining rapidly since 1990. At that time, Myanmar fishery sector has effectively performed for raising aquaculture production capacity in different States and Regions. Recently, freshwater aquaculture is into commercial production of over 20 species and nearly 10 species of indigenous species were successfully done at experimental scale. Most of the Myanmar people live in rural areas and they can easily access fishery resources in their place for daily food needs and create jobs. The aquaculture sector has been performing priority projects towards sustainable management of marine and freshwater fisheries to address overexploitation and climate change impact.

Myanmar DOF has initiated projects to ensure food security, food safety, and environment friendly and sustainable development of aquaculture sector through cooperation with local, regional and international organizations. Myanmar aquaculture sector would like to need improve aquaculture value chains, environmental sustainability, and competency of staff. This could be done through application of advanced technology and cooperation with international organizations through research and development programs for seed production in marine finfish.

Introduction

The Government of the Union of the Myanmar has a total land area of 676,580 km^2. It has a total coastal line of 2,832 km. It has four major river systems, including several large estuarine delta systems, as well as permanent and seasonal freshwater bodies with a total of 82,000 km^2 that provides habitat for a considerable diversity of aquatic species. In 2017-2018 fiscal year, the total production of fish was 5.87 million metric tons in Myanmar. In this period, the production of freshwater fish was 2.72 million metric tons and the production of marine fish was 3.15 million metric tons. The exported amount of fish and fishery products was 0.57 million metric tons and the value of which was 711.72 million in USD. It was exported to 46 different countries. The exported amount was 10% of the total production of fish in Myanmar in this period.

The fisheries sector has advocated ensuring food security, food safety and sustainable

development of fisheries sectors by conservation of fisheries resources in accordance with the fisheries law as policy. In order to aquaculture sectors is initiate conservation of indigenous species and conducting research and in breeding and culture of those species, allowing import of high quality fish/shrimp seeds/broodstock and producing genetically improved fish species, adoption of climate smart fish species and their related breeding and culture technique, collaboration with regional and international organizations for preventing and controlling of fish/shrimp disease, cooperation with public, private and local/international organizations for the promotion of sustainable aquaculture activities, and Strengthening the development of environmental friendly aquaculture system such as Good Aquaculture Practices to align with ASEAN guideline according to EU market requirement.

Sustainable aquaculture

Freshwater aquaculture

Freshwater finfish farming commenced in 1953. Currently, over 20 species of freshwater fishes including common carp, indian major carps, Chinese carps, tilapia, *Pangasius* and walking catfishes and Pacu are being cultured in 27 government hatcheries stations. Freshwater fish culture is dominant aquaculture and 95 percent of Myanmar aquaculture are major carp such as rohu, mrigal and catla. Aquaculture provided animal protein intake of population and job opportunities along value chain. Rohu (*Labeo rohita*) has become key aquaculture commodity. These major carps are widely distributed across the Indian sub-continent, emcompassing countries such as Pakistan, India, Bangladesh, Nepas and Myanmar. These major carps also had been widely breeding in the whole Myanmar aquaculture (statistic of Myanmar, 2009). Major producer countries include India, Myanmar and Bangladesh (FAO, 2009).

In addition since the 19th century Myanmar aquaculture division has done fish restocking into a number of water bodies to replenish the depleted wild populations and enhance the freshwater fisheries resources (statistic of Myanmar, 2008). Therefore, Network of Aquaculture Center in Asia-Pacific (NACA) supported scholarship for master degree to identify genetic diversity of hatchery and wild populations of *Cirrhinus cirrhosis* (Bloch, 1795) at Kasetsart University in Thailand. The outcome of this master degree study was to obtain levels of genetic diversity of hatchery and wild populations of mrigal, and to provide recommendations for the genetic management and sustainable use of genetic resources of mrigal (Aung, 2009). After this study, Aquaculture Division was tasked to produce quality fish seed not only for genetic improvement and selective breeding program of rohu but also broodstock management of other hatcheries brood fishes following the result on genetic status of wild and hatchery populations of mrigal in Myanmar.

These hatchery stations conduct research studies on potential indigenous species in collaboration with international organizations. Hatcheries station want to research on local species but difficult to get budget for research. Ice cold areas in northern part of the country have less developed fish culture due to limitation of – topographical favorable condition, remoteness of mountain and difficult access. Formerly, consumers bought fish products from other area for food security with high price. Recently, aquaculture sector has established backyard hatchery to produce seedstock of common carp, Chinese carps and tilapia for grow-out culture and stock enhancement.

Marine aquaculture

Most common and prioritized species are the commercially important giant freshwater prawn, *Macrobrachium rosenbergii* and *Penaeus monodon* (tiger shrimp). Shrimp ponds are mostly along the coastal region since 1980 with trap and hold system. Most of the prawn farmers practiced the polyculture system stocked with freshwater prawn and fish to minimize the operational cost. Myanmar DoF and SEAFDEC/AQD early started mangrove friendly shrimp culture in 2002 with few farmers due economic constraints. Now a day, three types of culture systems are practiced: extensive, extensive plus and semi-intensive. DoF encourage fish and shrimp culture in every states and regions for environmentally non-degradation and technically appropriate. Farmers buy brood stock from Bay of Bengal and Andaman Sea. Government Hatchery stations and private hatcheries are faced with the problem of insufficient amount of breeders to produce in every years. Last year, farmers exported prawn/shrimp breeders to Thailand and other countries. Recently, farmers have been importing prawn/shrimp seeds from other countries because of the difficult requirements in local hatcheries and its high price. Since 2000, white shrimp started to be cultured and permitted include reasonable documentation in domestic water. DoF implemented standard operating procedure to prevent Tran-boundary aquatic animal diseases (TAADs). Trans-boundary aquatic animal diseases can rapidly impacts on biodiversity in the natural resources that limit the development and sustainability of the fisheries sector through production losses and other negative consequences such as direct and indirect impacts on livelihoods, increase operating costs, restrict trade, reduce market share and result in investment losses.

Marine species in Myanmar, mainly sea bass, groupers and red snapper are well develop in coastal aquaculture. They are formerly cultured using fry and juvenile caught from the wild. Now a day, grouper and seabass are hatched from DoF hatcheries (Myeik and Chaung Thar) and private farms. Commercial scale Grouper net cages culture are being practiced at Kyun Su Township (Myeik area) in Taninthayi Division. Grow-out culture of seabass is an on going activity and expected to have development potential in the near future.

Meanwhile, mud crab fattening has become the booming industry as domestic consumption and export demand are growing rapidly. At the same time, supply of crab juveniles from nature is decreasing due to over exploitation, habitat deterioration caused by human impact. There is a need to do more research and extension work for the dissemination of mud crab culture techniques to local small scale farmers and the conservation of mud crab resources, such as by setting up of protected ares of no crab fishing zone or conservation of mud crab habitats such as mangrove. At present, make smart company has extended the culture area of *Eucheuma cottoni* and also constructed processing plant and storage building to accommodate production of 8,000 tons per year.

Management practices

Myanmar is one of the member of ASEAN countries, The Department of Fisheries of Myanmar already initiated Good Aquaculture Practices (GAqP) as ASEAN standard in fish and shrimp farming since 2011. DoF established the Directives and Regulation for prohibiting the use of chemical in aquaculture for food safety. At present, fisheries communities are more

interested in GAP guideline according to the market requirements. Indeed, aquaculture sectors try to practice GAP fish/shrimp/crab farming in the whole country.

From Farm to Table Approach in Aquaculture Products for Export responsibilities are fallowing with monitoring and inspection. Monitoring plan will be performed in fish farms as follows:

(1) surveillance of fish farm to examine disease infection and water quality,

(2) quarterly update on the awareness of the GAqP guideline in hatchery stations; and

(3) monthly sample collection in fish farms for examination under the National Residue Monitoring Plan.

Inspection plan implemented on fishery products in the lab are as follows:

(1) pathogenicity test to detect the presence of Viruses/Zoonotic Parasites on fishery products and live organisms for export,

(2) microbiological tests including for *Escherichia coli* and other coliforms, *Salmonella*, Total Plate Count, and

(3) analysis for the presence of chemical residues, veterinary drugs, contaminants and histamine.

Research

Nowadays, *Silonia silondia*, giant butter catfish (Nga Myinn), is a potential species for culture considering its demand in local markets and for being rare in the wild populations. There was a collaborative study on *Silonia silondia*, between the Department of Fisheries of Myanmar, Yangon University, and Tokyo University of Marine Science and Technology in Japan. Samples were collected monthly along the Ayeyarwady River for one year to determine genetic diversity. The samples were taken to Tokyo University for age determination based on growth rate, body length, and gonad weight. Samples were also reared, using developed broodstock management techniques, in two hatchery stations under the government.

Resource enhancement

In the field of aquaculture, a total of 48,672 fish and shrimp farmers were involved in various aquaculture systems. Since Myanmar's aquaculture is mainly based on pond culture system and mostly male labours are working in fish/shrimp ponds. Land use for extension of fish pond is permitted by local authority. Size of fish pond varies from less than one hectare to 40 ha depending on geographic situation Productivity also varies 1,500 kg to 5,000 kg/Acre/year depending on type of culture operation. Twenty-seven hatcheries under DoF and 37 private hatcheries has managed to produce freshwater fish fry and fingerling for stock enhancement as well as for local consumption. According to DoF, hatcheries release the hatchery bred fish seeds into open bodies of water.

In order to maintain sustainable catch from inland fisheries, fishery stations have conducted yearly stocking of quality fish seed and juveniles into inland water bodies such as rivers, lakes, man- made reservoirs, canals, paddy fields and inundated areas. In the coastal area, shrimp hatcheries release hatcheries seeds into the ocean every year but cannot do so for marine fish seeds. Myanmar aquaculture sector lacks production techniques for marine finfish.

Mangrove forest protect and re-plantation program, collaboration with Forest Department, Local authority, NGOs and Local people. Other conservation and adaptation measures of freshwater fisheries are maintenance or reconstruction of water channel of leasable fisheries and mangrove re-plantation in the leasable fisheries. Department of Fisheries is collaborating with the fishers and fisheries stakeholders to conserve the freshwater resources and habitats. Capture based culture system is collecting the fingerlings of indigenous species or commercial species in the leasable fisheries and nursing in the fish pond or main channel of the leasable fisheries. While they grow up, they are released in the flooded area or rice field during rainy season. This system is favorable for the conservation of indigenous species or commercial species and for the promotion of fish production.

Biofloc and aquaponic techniques are performed by the aquaculture sectors but not in fisheries communities. Disease infection mostly occur in fish farms during the hot season. Lime, urea, zeolite/ dolomite, salt and T-super are used during pond preparation and to control disease. Currently, 70% of fish farms follow recommendations by the aquatic animal health disease section. The aquaculture division should be able to produce sufficient amount of prawn/shrimp seed for the local farmers with the implementation of Better Management Practices on biosecurity and aquatic health management, monitor persistent and emerging parasitic/bacteria disease in fish farms, and improved brood-stock management by vaccination to maintain aquatic animal health.

Conclusions

Aquaculture sectors had released hatchery bred quality fish seeds into the freshwater fisheries resources but has difficulty on the availability on data collection input and output results. For the sustainable development program, most of the project coordination and collaboration are with the Aquaculture Division. The aquaculture sector is sustainable if fisheries communities followed regulations, directives, guideline and fishery laws in Myanmar.

References

Aung, O. 2009. Genetic Diversity of Hatchery and Wild Populations of *Cirrhinus cirrhosus* (Bloch,1795) in Myanmar

FAO. 2009. Cultured Aquatic Species Information Programme- FAO Fisheries and Aquaculture Department [online].

Fisheries Department. 2008-2009. Fisheries Statistics. Ministry of Livestock and Fisheries, Yangon, Union of Myanmar.

Fisheries Department. 2017-2018. Fisheries Statistics. Ministry of Agriculture, Livestock and Irrigation, Yangon, Union of Myanmar.

Trends in the Major Aquaculture Food Fish Production in the Philippines

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Abstract

Predictability of food fish derived from the aquaculture sector is a pragmatic concern for the society at large. In this paper, the trend in aquaculture production is presented, with emphasis on the two major food fish species, milkfish and tilapia. Particular interest is on the assessment of observed over-all decline on the rate of output generation in the recent years and its major cause. Accordingly, the impacts on supplies and estimates on the needed catch-up growth rates for the milkfish and tilapia sub-sectors (excluding municipal inland fisheries) are explained. Adjustments currently being implemented by the milkfish and tilapia farming sub-sectors are discussed. Finally, selected prospects related to farming site expansion, emerging farmer-oriented information needs and quality of critical inputs are discussed. In the context of this important occasion, the International Workshop on the Promotion of Sustainable Aquaculture, Aquatic Animal Health and Resource Enhancement in Southeast Asia (SARSEA), interventions offered herein are deemed relevant to the greater Southeast Asian region as well.

Introduction

Macro production trends in the fisheries sector

The increasing contribution of aquaculture in the overall fisheries production in the Philippines reflects the global trend. The local aquaculture contribution to the total fisheries output has grown from 36 % to 53 % over the last 20 years (**Figure 1**).

The UN-FAO 2018 SOFIA Report (State of the World Fisheries and Aquaculture) mentioned that the share of aquaculture output to the world fisheries production is at 46.8 %.

According to the UN FAO 2018 SOFIA, the Philippines is ranked in the world aquaculture as follows (based on 2016 data):

a. 3rd in seaweeds, next to China and Indonesia;

b. 5th in fish production from marine and coastal, after Norway, Indonesia and Chile;

c. 9th in marine crustacean production, after Mexico, Thailand and Bangladesh

d. 11th in total aquaculture output, excluding seaweeds, next to Chile and Myanmar

According to PSA (2018), the Philippine aquaculture output was 2,304,122 tons, valued at Php 110.329 billion or approximately US$ 2.099 billion (Php 52.554: 1 US$ June 01, 2019; **Figure 2**). The total value of output indicates food aquaculture items such as fish, and crustaceans represents more than 80% of the total value. A summary graph and table below (**Figure 3**) indicate the relative economic significance of each commodity based on its raw form or ex-farm valuation. It is important to note that while tilapia and milkfish are relatively cheaper products (US $ 1.96 and 1.48 per kilo ex-farm), their combine massive output comprises almost 60% (approx. US $ 1.185 billion) of the total value of outputs in 2018 (**Figure 4**).

Species, farming systems and output

The species of farmed fish in Philippine aquaculture is fairly diverse (**Table 1**). Meanwhile, the relative contribution of these species to the total output over the years (e.g. 2008 versus 2018) has not dramatically changed, albeit substantial enough in response to externalities and pursuit production efficiencies.

There is a distinct dominance of species relative to farming environment based on historical 2008 and 2018 PSA datasets. Tilapia, milkfish and seaweeds remain the primary species in the freshwater, brackishwater and marine environments, respectively. The 2018 data indicates increase in the total output in the brackishwater sub-sector by 21,726 tons, brought by milkfish, oysters, mud crab (or mangrove crab) and notably tilapia. The marine sub-sector meanwhile contracted by 150,041 tons mainly due to seaweeds, however, milkfish farming in cages has increased by 37,729 tons (PSA, 2008 and 2018 data sets).

Growth in oyster, mangrove crab and white shrimp are expected to increase in the coming years. Technology transfer efforts by SEAFDEC/AQD and DA-BFAR on mangrove crab farming in key provinces such as Catanduanes, Quezon, Iloilo are in full-swing. Two new private oyster hatcheries (i.e. Arton Farms and ASIN Inc.) which benefited from SEAFDEC/AQD training are now poised to go on commercial scale operations. Furthermore, investments on intensive white shrimp farming in Pangasinan, Southern Luzon and Southern Mindanao are progressing.

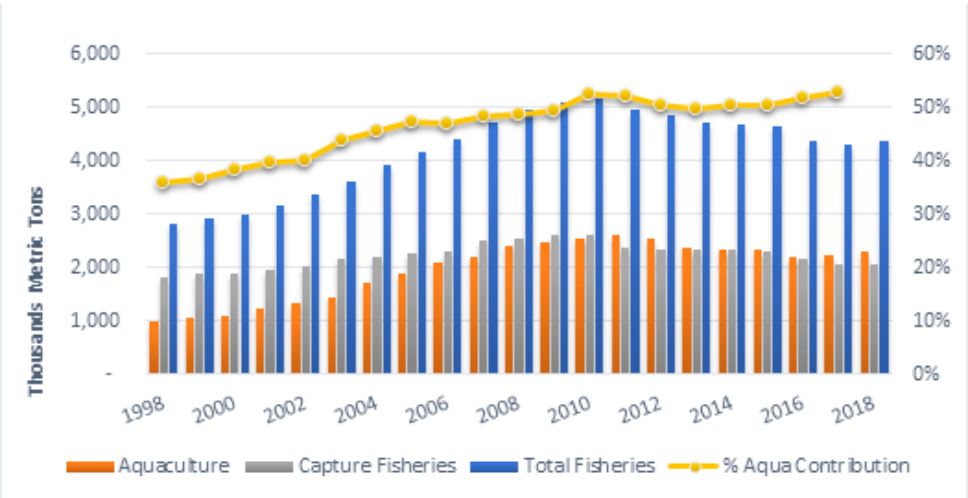

Figure 1. Fisheries production and aquaculture contribution from 1998-2018

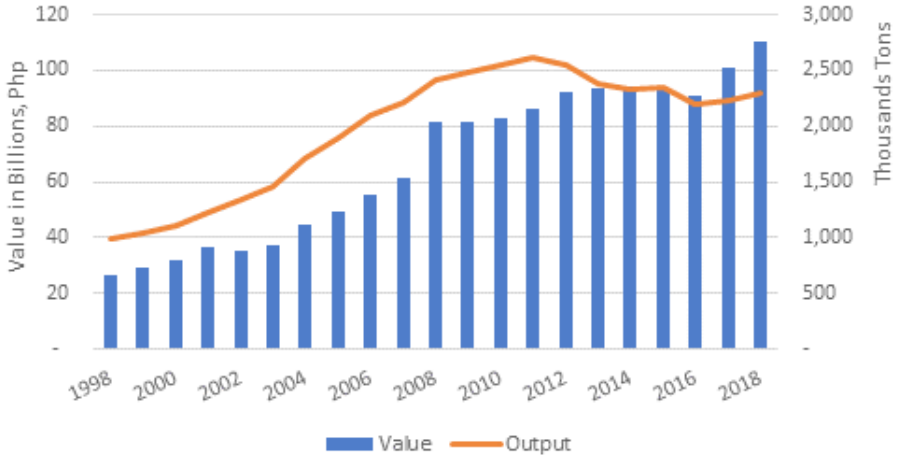

Figure 2. Value and output of total Philippine aquaculture

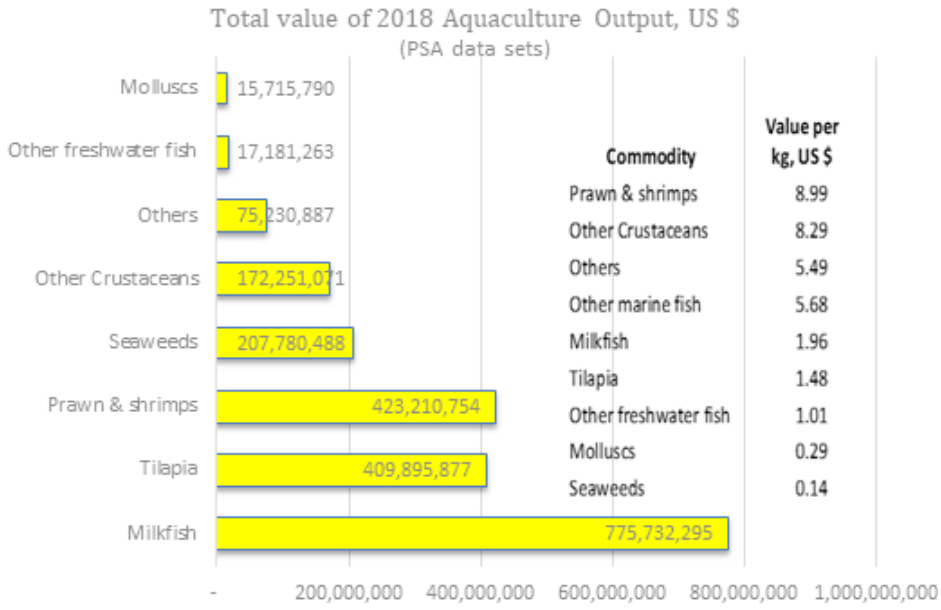

Figure 3. Total value of 2018 Aquaculture output.

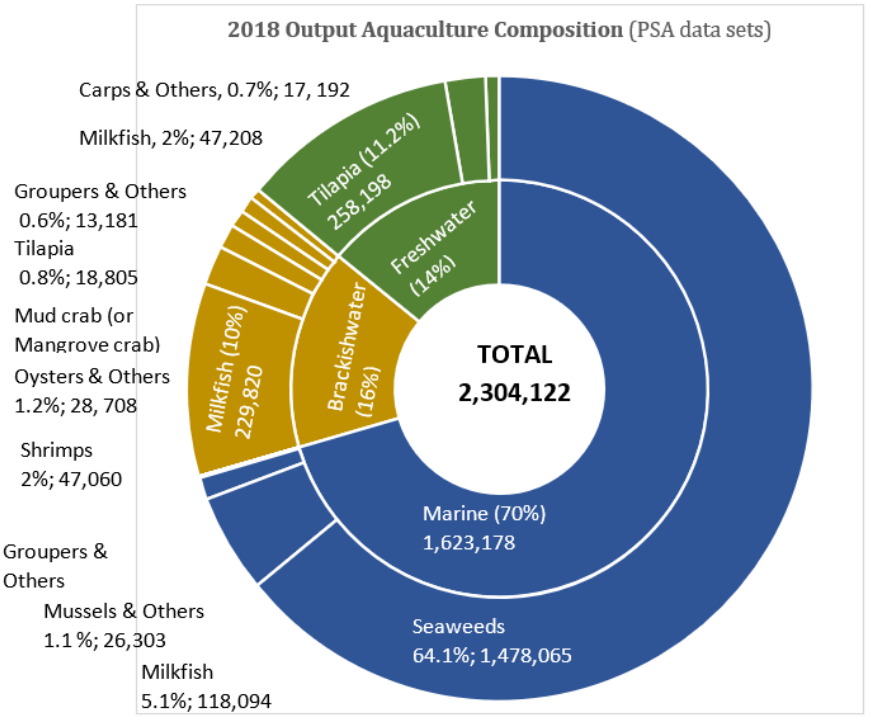

Figure 4. 2018 Output Aquaculture Composition (PSA dataset)

Table 1. Farmed fish species in the Philippines

Group	Species
Freshwater	Tilapia: *Oreochromis niloticus*, *O. mossambicus*, and its hybrids
	Carps: *Cyprinus carpio*, and *Aristichthys nobilis*
	Catfish: *Clarias gariepinus* and its hybrids
	Others: Mudfish *Chana* spp., Gourami species (Giant and Common)
Diadromous	Milkfish: *Chanos chanos*
	Eels: *Anguilla marmorata* and *A. bicolor pacifica*
Marine	Grouper: *Epinephelus coioides*, *E. fuscoguttatus*
	Others: Sea bass *Lates calcarifer*, Pompano *Trachinotus blochii*,
	Siganid: *Siganus guttatus*, *S. vermiculatus*,
	Snapper: *Lutjanus* spp.

Group	Species
Crustaceans	Tiger shrimp: *Penaeus monodon*
	Endeavor prawn: *Metapenaeus ensis*
	Whiteleg shrimp: *Litopenaeus vannamei*
	Mangrove crab: *Scylla serrata* and *S. olivacea*
	Giant freshwater prawn: *Macrobrachium rosenbergii*
	Spiny lobster: *Panulirus* spp.
Mollusks	Green mussel: *Perna viridis*
	Oyster: *Crassostrea iredalei* and *Saccostrea cucullata*
	Others: Pearl oyster *Pinctada* spp. and Abalone *Haliotis* spp.
Seaweeds	*Eucheuma/Kappaphycus*
	Gracilaria spp.
	Sea grapes *Caulerpa racemosa* and *C. lentillifera*

On the other output of marine (e.g. grouper and pompano) and other diadromous (e.g. seabass, siganid) species are expected to remain low due to limited investments on hatchery.

Trends in the major farmed fish production

Nowadays, more than half of fishery products served on tables worldwide are farm-raised (51 % in 2015), according to the UN FAO 2018 SOFIA. The country data meanwhile shows a steady increase to that effect growing at 1.1 % per year on the average for the last 10 years. In PSA2018 data, the local aquaculture provided food fish in the tune of 826,064 tons or 27.9 % of the total fisheries (**Figure 5**). The explanation on what drives local consumption of farm-raised fish is sensitive to the culinary culture of the population. Per observation, Filipinos prefer live tilapia and would not likely buy its frozen counterpart. This apparently simple issue on product presentation has a strong influence on production in general, as the market for (stock-piled) frozen tilapia is almost zero at this point. Nevertheless, with the global trend as the benchmark and participation in the seafood world trade are being considered, the Philippine food aquaculture will have to significantly increase its output in a sustainable manner.

The graph (**Figure 6**) depicting two decades (1998-2018) of food fish aquaculture production clearly shows that milkfish and tilapia are the primary output generators (PSA datasets). In 2018, milkfish and tilapia contributed 48 % (395,130 tons) and 34 % (277,006 tons), respectively in that particular year. On the other hand, long-term local data shows a steep decline in the rate of growth in the entire food aquaculture (**Figure 7**). From a high of 7.98 % average increase between 2000 and 2005, it contracted to 1.19 % between 2012 and 2017 (PSA datasets). This observation should be taken seriously and demands pragmatic interventions from the entire stakeholder base.

Evaluation of datasets on milkfish and tilapia farm outputs pertaining to years 2000–up to the present affords us perspectives on the trends (**Table 2**).

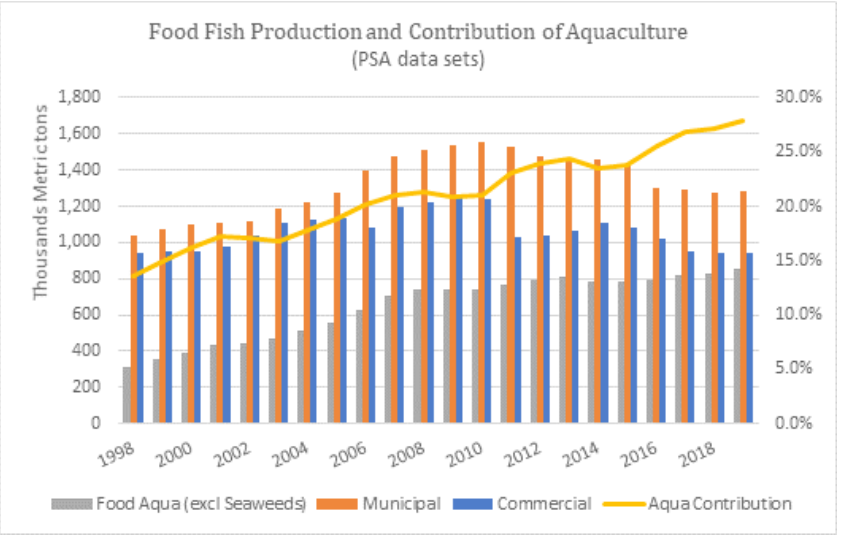

Figure 5. Food fish production and contribution of aquaculture from 1998-2018

Primarily we are concerned about the rate of growth in tilapia and milkfish outputs as a sound metric of farmed fish sufficiency. Surprisingly, the average annual output growth in the years 2012–2017 is only 31 % (8,938 tons) of the years 2000–2005 (28,021 tons). Furthermore, the percentage increase of the average annual output from 2006–2011 (539,581 tons) to 2012–2017 (609,566 tons) was barely 13.0 %. These pieces of information corroborate the downward trend of the growth of output which can easily slipped unnoticed by merely looking at the increasing absolute value of tons of farmed fish produced year by year.

The apparently diminished averages of growth in all major farming systems beyond 2000–2005, for both species lends many important insights. Brackishwater ponds generate 57 to 60 % (227,000 to 243,000 tons) of the total milkfish outputs (PSA 2015 to 2018 datasets). The narrow rate of increase is primarily inherent to the available areas. Conversion of mangroves for fishpond use are no longer allowed as a consequence of ban in cutting mangrove vegetation (RA 7161 of 1991). Vast areas of traditional brackishwater fishponds are leased/bought by marine cage farm operations to ensure production of cage-appropriate size milkfish fingerlings (>10 cm TL). Loading of one (1) unit 15 m diameter, HDPE floating milkfish fish cage (40,000 to 50,000 pcs) per cycle, would require fingerling output from two (2) hectares brackishwater fishpond. This development is expanding in Davao and Northern Mindanao provinces. Freshwater fishponds generate 46 to 48 % (142,00–155,000 tons) of the total tilapia output in the years 2015-2018 (PSA datasets). It is reasonable to surmise that new expansive tilapia fishpond development is very limited. Instead, what is observed is that real-estate/housing development projects are fast converting flat agricultural lands otherwise used for tilapia or rice/vegetable farming. According to tilapia sector leaders, consolidation of fishponds holdings is the current trend particularly in Pampanga in Central Luzon region. Consolidation of up to 50 hectares and above is motivated by the intent to achieve economically competitive scale of operation.

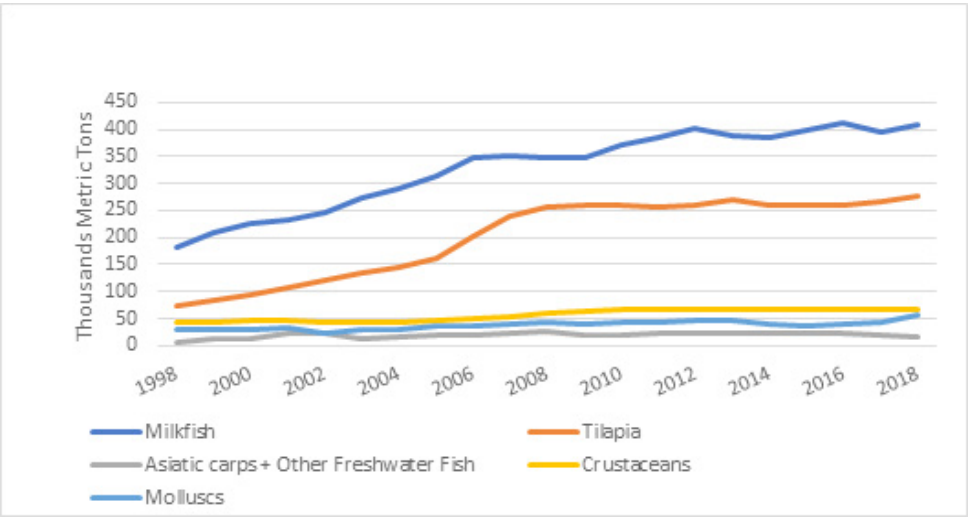

Figure 6. Philippine Aquaculture Production Trend, excl. Seaweeds (PSA data sets)

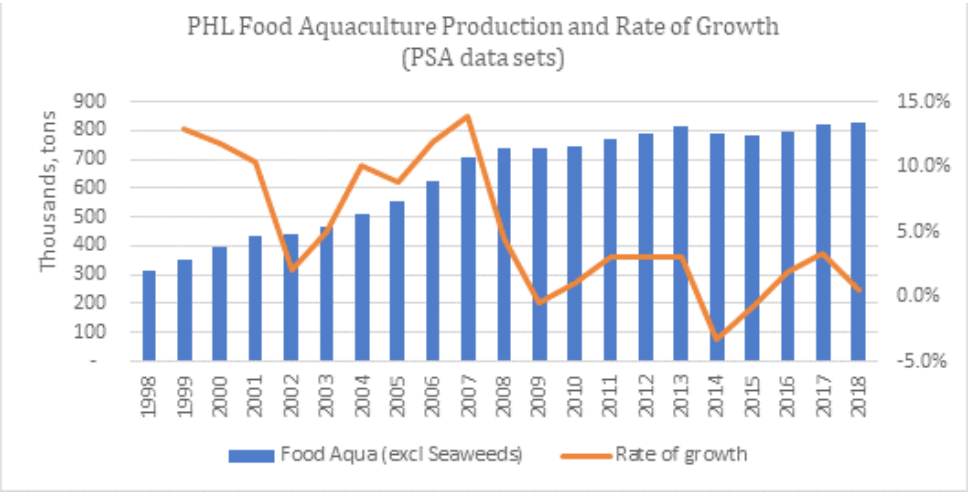

Figure 7. Philippine food aquaculture production and growth rate from 1998–2018 (PSA data sets)

Table 2. Growth and Output Generation in Milkfish and Tilapia Farming Systems, Philippines, 2000–2017 (PSA dataset)

Commodity	Indicator Farming systems	Average Growth%		
		2000–2005	2006–2011	2012–2017
Milkfish	Brackishwater ponds	5.4	1.1	1.4
	Marine cages	53.9	19.8	4.5
	Freshwater pens	43.4	2.7	3.0
		Average Annual Total Output, tons		
		2000–2005	2006–2011	2012–2017
	Brackishwater ponds	197,765	219,166	234,397
	Marine cages	13,502	61,079	102,737
	Fresh water pens	15,729	2,116	27,024
		Average Change in Annual Output, tons		
		2000–2005	2006–2011	2012–2017
	Brackishwater ponds	9,177	2,380	3,239
	Marine cages	4,651	8,631	3,989
	Fresh water pens	1,411	608	657
		Average Growth %		
		2000–2005	2006–2011	2012–2017
	Freshwater ponds	14.2	10.4	1.5
	Freshwater cages	9.0	6.3	-0.2
	Freshwater pens	60.7	9.3	-3.4
Tilapia		Average Annual Output, Tons		
		2000–2005	2006–2011	2012–2017
	Freshwater ponds	63,944	133,361	143,184
	Freshwater cages	47,824	78,647	81,981
	Freshwater pens	6,804	20,211	20,243
		Average Change in Annual Output, tons		
		2000–2005	2006–2011	2012–2017
	Freshwater ponds	7,274	9,610	2,153
	Freshwater cages	3,969	3,918	-347
	Freshwater pens	1,540	1,495	-752
Summary: Milkfish and Tilapia outputs		**2000–2005**	**2006–2011**	**2012–2017**
Ave. annual output, tons		345,560	539,581	609,566
Ave. annual change, tons		28,021	26,643	8,938

To date, official statistics on brackishwater fishponds (134,739 ha) and freshwater fishponds (16,603 ha) were based on survey conducted between 2005 and 2010 by the Philippine Statistics Authority (PSA). The said agency is about to release the results of "Updating of List of Aquaculture Farms (ULAF)" project. It is important to note that while the area of freshwater fishponds is only 12 % that of brackishwater ponds, the output of the latter approximates up to 60 % of the former. The tilapia farming community attributed significant increase in farm productivity with the introduction of high-yielding Nile tilapia strains such as the Genetically Improved Farmed Tilapia (GIFT) strain which was launched in 1993, after almost a decade-long research in the Philippines (ADB, 2005). Meanwhile, introduction of genetically improved milkfish is yet to be realized. Per hectare output for each crop of pond-raised tilapia in Pampanga is between 5.61 to 6.04 tons (PSA datasets), while the national average for milkfish pond farming is in the range of 0.72 to 1.05 tons (PSA datasets), or 1:5-6 in favor of tilapia ponds.

Stagnation of milkfish outputs from freshwater fish pens were glaring in the years 2006 to 2017, while the reverse is true for tilapia. This can be explained by, first, the significant shift to marine fish cage farming, which now generating 3.8 times more milkfish than from freshwater pens (PSA datasets, 2012–2017). Second, data suggests probable shift in favor of tilapia over milkfish in freshwater pens as shown in the ratio of milkfish to tilapia output: 2.3:1 in 2000–2005 declined to 1.35 in 2012–2017. This is perhaps a spill-over effect of the above-mentioned first cause, as poly-culture of milkfish, tilapia and Asian carps is the norm in freshwater pen systems (Ortega, 2013).

Please note that resource use restrictions are being implemented by concerned government (national and local) agencies to balance the utilization of mixed-used inland bodies of water. A precautionary blanket ceiling of not more than 10 % of the lake or river surface area can be utilized for aquaculture is clearly specified in the amended Fisheries Code (Sec. 51, RA 10654). Furthermore, environmental laws, (e.g. RA 7586 and its expanded edition RA 11038), designed to protect areas of national interest also regulates the expansion of aquaculture in many lakes covered under these legislations. Taken together, these put a cap on expansion of freshwater fish pen and cages, however, not necessarily intend to limit sustainable intensification.

The rise of fish cage operations in marine environments for milkfish and in freshwater for tilapia is remarkable. PSA 2018 data indicated that fish cage grown milkfish (108,237 tons) and tilapia (85,440 tons) provided 27 % of the total output for these commodities. The increase is attributable to economic and technological efficiencies. Modern circular (15 to 18 m. dia.) HDPE-made cages is becoming common, phasing out the bamboo or galvanized iron (GI)-framed square cages (5 m × 5 m up to 18 m × 18 m) in milkfish farming. Output using circular HDPE cages ranges from 22 to 30 tons per crop realized in 5 to 6 months. This output is 1.6 to 1.9 times more than a typical 18 m x 18 m x 10 m GI framed milkfish marine cage in Pangasinan. In tilapia, either GI or bamboo framed cages remains the norm in areas such as Taal Lake and Magat Dam. A typical 10 m × 10 m × 10 m GI-framed tilapia cage in Taal Lake generates at least 10 tons every 6 months cycle.

Accordingly, fishpond development is more expensive, estimated at US$ 19,028 to 27,700 per hectare of raw land versus procurement and deployment of a 15 m diameter HDPE cage, at US$ 7,600 (direct

importation and own assemble) to US$ 11,790 (local vendor package). Cost of tilapia fishpond development is relatively less expensive (20-30%) by some estimates than brackishwater ponds, while cost to fabricate GI-framed cages ranged between US$ 1,300 and US$ 1,600 per unit (DA-BFAR-300 Fish cage Project, 2018).

Growth trajectory shows intensification, measured by the average annual output of marine fish cages, continued to expand compared to its tilapia counterpart in lakes. Comparing year 2000 and 2017 PSA data indicates marine fish cage output grew 39.5 times while barely 2.1 times for tilapia cages. In fact, tilapia cage annual contribution contracted by 347 tons on the average, while milkfish marine cages added 3,989 tons between 2012 and 2017. This is an indication that upper limits of major lakes (e.g. Taal Lake in Batangas, Lake Sebu in South Cotabato, Lutayan Lake and Buluan Lake in Maguindanao) that are currently utilized in tilapia cage and pen operations has been reached. Nevertheless, while coastal and nearshore farm sites generally have a higher carrying capacity due to "cleansing" effects of tides and currents and vast surface area, this system is also vulnerable in exceeding its carrying capacity, as evidenced by recurring and relatively new fish kill incidents in Pangasinan and Misamis Oriental marine farm sites.

Finally, the combined average of added milkfish and tilapia biomass annually contracted by 30.8 %, down to 8,938 tons from 28,021 tons between 2000–2005 versus 2012–2017. Discussions on the impact of this downtrend, underlying contributing factors and some investments/interventions needed are discussed in the following sections.

Impacts on farmed fish supplies

Estimates of demand and supply gap presented in **Tables 3** and **4** are based on the following key assumptions:

a) Fish-eating population is based on the PSA population projection (high assumption) for the specified years then subtracting 30% and 50 % of the 0 to 4 and above 80-year old groups, respectively;

b) Demand per commodity is the product of fish-eating population multiplied by the per capita consumption (Consumption of Selected Agricultural Commodities in the Philippines Vol. 2, PSA, 2017). Furthermore, it is assumed that half of the fish-eating population are urban dwellers.

It is clear that significant amount of supply gap will have to be filled-up by 2025 and onwards should the current dismal rate of growth persist. Based from the offered growth estimates, milkfish and tilapia should increase by 40.7 % (555,988 tons) and 65.9 % (459,560 tons) by 2025, from their respective 2018 base amount. This straight forward assessment reflects the estimates provided in other rather more elaborate available benchmarking documents over the same time horizons (**Table 5**).

A perspective on the probable causes of decline

The author perceives that a pervasive factor, that is the occurrence of natural calamities, is the primary caused and exacerbated the rapid decline across the aquaculture production systems in the

Philippines. Data on natural calamity incidents that impacted the Philippines from the International Disasters Database (www.emdat.be) was filtered based on the following criteria and shown in **Table 6** and **Figure 8**. More than 20,000 total affected persons;

a) Impacted multiple provinces, particularly areas with high aquaculture output;

b) Climatological, meteorological and hydrological incidents only.

Table 3. Estimated aquaculture food fish demand, Philippines 2018 and projections

Indicator Commodity	Per capita, Comsumption, kg		Base 2018 production, tons	Fish Eating population	
				2025	2030
	All Group	Urban Group		110,327,391	117,324,563
				Commodity specific demand, tons	
Milkfish	4.193	5.291	395,130	523,205	556,388
Tilapia	3.950	3.905	277,006	433,340	460,823

Table 4. Estimated aquaculture food fish supply gap and required catch-up growth rates, Philippines, 2017 and projections

Indicator Commodity	Commodity Average annual growth, % 2012-2017 (PSA datasets)	Estimated commodity supply gap, tons		Fish Eating population	
		2025	2030	2019–2025	2026–2030
Milkfish	1.69 %	78,893	73,241	5.0 %	1.5 %
				32, 783 tons	42, 569
Tilapia	0.69 %	142,675	159,991	7.5 %	2.0 %
				26,226 tons	46,575 tons

Table 5. Key industry scenario planning references, Philippines, 2021-2035 projections

Years	Aquaculture Futures DOST/PCAARRD-WorldFish, 2016		Comprehensive National Fisheries Industry Development Plan, (DA-BFAR 2008)	
	2021–2025	2026–2035	2021–2025	2026–2035
Milkfish	2.6-5.0 %	1.4-5.0 %	5.0 %	No data
Tilapia	4.2-8.1 %	2.3-8.1 %	5.0 %	No data

Table 6. Summary of natural calamities that impacted the Philippines, 1998-2017

Natural calamities	Number of incidents over time periods		
	1998-2005	2006-2011	2012-2017
Storms	23	35	26
Flooding	4	15	9
Drought	2 (1998 & 2002)	1 (2007)	1 (2015)

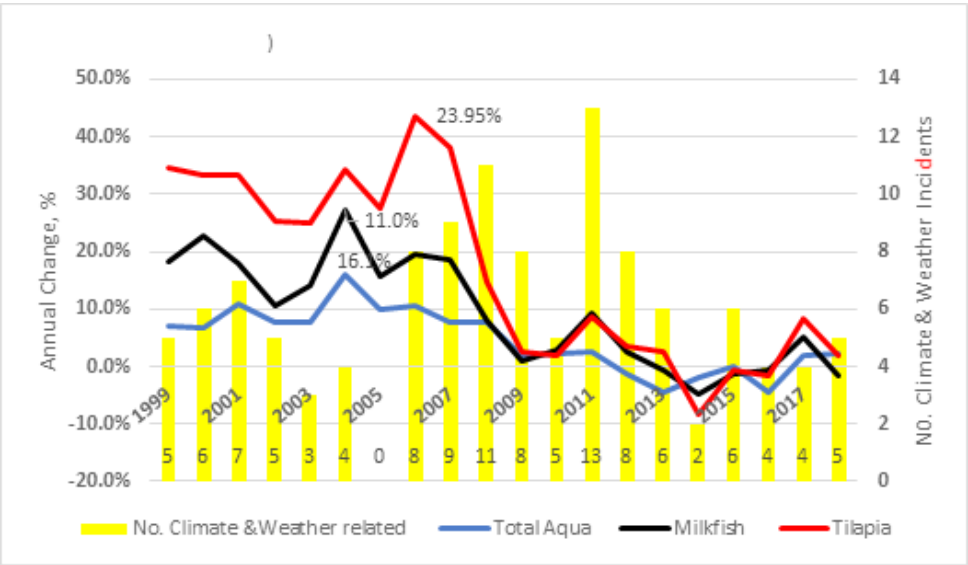

Figure 8. Twenty- year Rate of Change (%) in Aquaculture and Occurrences of Selected Natural Calamities (PSA datasets and www.emdat.be U.C. Louvain Belgium)

It is clear from the graph below that aquaculture output tends to regain in the years immediately after zero or low occurrences of natural calamities (e.g. 2004, 2006, 2011, 2015 and 2017). Conversely, contraction in outputs are evident in years following the highest number of natural calamity incidents. Furthermore, it appears that the years 2006 and 2007 were the tipping point, wherein the high growth rates previous to this reference points have yet to be surpassed to date.

One can easily imagine the financial devastation to farm operators and its crippling effect on investments supposedly for farm expansion. Data on the economic value of damages on fisheries or aquaculture must be collated from multiple sources and consequently analyzed. Needless to say, an in-depth multi-perspective study will definitely add to our understanding particularly on sectoral investment planning, disaster risk adaptation, and impact mitigation, among others.

Selected prospects

Expansion and alternative areas

Private investments are the primary engines that drives the Philippine aquaculture and the entire fisheries sector at large. It is remarkable that despite the impact of pervasive and cyclical natural calamities, local and foreign investments in aquaculture remains in the country. Based on the data shown in the previous section (D. Impacts on farmed-fish supplies), the future value of the 2025 demand for milkfish (523,205 tons) and tilapia (433,340 tons) is estimated at US$ 1,180 billion and US$ 765 million, respectively. As such, there is a compelling reason to invest in the Philippine aquaculture from business and social obligations perspective despite the risks imposed by natural forces.

From the previous section (C. Trends in the major farmed-fish production), the integration of marine fish cage and traditional brackishwater fishponds into a consolidated, modern and highly productive production chain is the future. Similar trend has been observed in the case of tilapia farming in freshwater environment, however, the over-all scale is smaller than the former. These developments are sensible from the standpoint of productivity, economic efficiencies and response to perennial bouts with natural calamities. It was also raised that both marine and freshwater environments are vulnerable to pollution-induced or naturally-caused fish kills. In the context of disaster risk impact reduction, it is imperative that exposure of food aquaculture sub-sector to hazards must be minimized, if not addressed as in the case of pollution related fish kills.

Sometime in June, 2019, DA-BFAR tasked the author to conduct a desk assessment in coordination with DA-BFAR Regional counterparts, on the potential milkfish mariculture expansion areas in the country. The said exercise shows that the Philippines has approximately 3,224 hectares of potential marine farm-sites, with a 25,643 cage capacity with a potential output of at least 1,0225,720 tons per year, based on the assumptions below, and a map was prepared accordingly:

a) One (1) cage unit is 15-18 m diameter HDPE cage, 8 m, net depth;

b) One (1) cage unit can produce 40-60 tons per year;

c) One (1) hectares surface area can accommodate eight (8) units of HDPE cage;

d) Only 20-50% surface of identified potential area will be utilized;

e) Not included are mariculture areas/ zones that are:

- Subject to environmental assessment/review;
- Areas already operating in full capacity (i.e. existing sites);
- Areas within NIPAS protected seascapes; and
- Previously identified areas deemed not productive.

The above-information clearly shows that the Philippines has sufficient marine areas to fill-in the immediate and long-term milkfish demand and alternative/ relocation sites to allow recovery of heavily utilized areas. The above estimated potential is almost 10 times of the current capacity, based on 2012-2017 average annual total output of milkfish marine cages (102,737 tons). Furthermore, this is by no means an exhaustive assessment of available expansion areas, considering the country has 184.6 million hectares of continental shelf (<200 m depth) (Philippine Fisheries Profile, 2016 DA-BFAR). The above assessment remains to be rigorously analyzed particularly from the so-called "climate-lens", value-chain perspectives and carrying capacity modeling. Nevertheless, it can be claimed that there is a sufficient expansion area for consideration of private investors and accordingly mobilize in coordination with the concerned local government units having jurisdictions over the areas and DA-BFAR for technical assistance.

Alternative sites for tilapia cage farming is warranted to augment outputs generated from freshwater fishponds and to afford down-scaling of operations on heavily utilized lakes. Particular interest is on Lake Mainit (14,000 hectares) in Agusan del Norte and Lake Naujan (11,000 hectares) in Mindoro Oriental. These lakes are currently not extensively used for aquaculture operations. Based on the Asian Development Bank documents, approximately 2,500 tons per year can be produced in a modest scale integrated tilapia project in Lake Mainit (ADB ADTA 4807, 2007). In addition, utilizing at least 5 % of the surface area of Lake Naujan could potentially yield 44,000 tons per year (i.e. 550 hectares, with 4 cages per hectare each producing 20 tons per year). Meanwhile, a maximum of 10 % of the surface area of a lake or river can be utilized for aquaculture is clearly specified in the amended Fisheries Code (Sec. 51, RA 10654). The combined rough estimates of production capacities of Lake Naujan and Lake Mainit represents 10.7 % (46,500 tons) of the projected demand for tilapia in 2025 (433,340 tons).

Research and development to build and disseminate user-friendly environmental modelling packages to estimate carrying capacity, determine best fish cage sites and other functionalities is needed in aide of sustainable aquaculture intensification both in marine and freshwater environments. The complementary use of geographic information systems for marine/lake spatial planning would also prove to be very useful. In addition, GIS-based technologies have been used for multi-parametric site suitability analytics in crop farming which can be extended to aquaculture.

Knowledge dissemination

Dissemination of better practices that have been proven by farmers and technologists

borne out of the so-called "new normal" of aberrant weather/climate conditions is important. DA-BFAR in partnership with innovative farmers, DOST-Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) and UN FAO Philippines developed a new set of knowledge products with a series title "Impact Management of Weather Systems on Aquaculture" (IMWS). Invited SEAFDEC/AQD experts also provided inputs during its development. This publication is eleven (11) part series which covers all farmed species and corresponding farming systems across all four (4) climatic conditions in the country. The IMWS series provides details on farmer-proven and scientifically-validated practical techniques to mitigate impacts of climate/weather disturbances and response to emergencies. Documentation and validation of such knowledge must continue and popularized.

Sustainable intensification in essence is producing more with less inputs. Along this line, UN FAO tapped the expertise of SEAFDEC/AQD and DA-BFAR on projects for efficient use of aquaculture feeds. Developed knowledge products namely, FAO Fisheries and Aquaculture Technical Paper 614 (2018) "Better management practices for feed production and management of Nile tilapia and milkfish in the Philippines" and FAO Fisheries and Aquaculture Technical Paper 583 (2013) "On-farm feeding and feed management in aquaculture", are highly recommended for dissemination.

Access and quality of inputs

The realized gains of the successful introduction of genetically improved tilapia strains must be sustained. DA-BFAR's GET-EXCEL tilapia strain, is one of the so-called GIFT-derived strain, is the one of the most utilized tilapia genetic material in the country today (DA-BFAR Tilapia Commodity Program, 2018). DA-BFAR on the average produces 6.8 million pieces of GET-EXCEL broodstock for distribution to both private (e.g. Central Luzon Registered Tilapia Hatchery Operators) and government hatcheries. According to DA-BFAR Tilapia Commodity Program, approximately 30 % of the entire demand for genetically tilapia fingerlings (i.e. 1.352 billion pieces total national requirement) are being supplied through its broodstock distribution program alone. Recently, DA-BFAR National Freshwater Fisheries Technology Center and other DA-BFAR Technology Outreach Stations have been investing on improved design of tilapia artificial incubation systems and broodstock conditioning ponds to increase the production of high-quality fingerling. Recently, in 2017–2018, farmers in Pampanga complained about the so-called "Tagalog" others called it "Bulugan" strain, which is basically a mongrel. This "Tagalog" or "Bulugan" strain was described as hardy to poor water conditions and can out-compete normal tilapias in feeding but very thin-long body. Per information, this mongrel strain came from tilapia hatcheries in Laguna de Bay and bought by farmers despite their knowledge of its unknown pedigree. This underscores the need for DA-BFAR, SEAFDEC/AQD, tilapia farmers and other partner institutions to address the need for genetically improved tilapia strains.

One of the known limiting factors in local milkfish production is the low hatchery capacity for milkfish fry production. Demand for milkfish fry estimates varies from a low 2.5 to a high of 3.6 billion pieces annually (DA-BFAR Bangus Development Plan, 2020–2024). Long-term investments have been drawn by the Philippine government to build central and smaller satellite hatcheries in partnership with local government units and existing

hatchery operators. SEAFDEC/AQD have been working with DA-BFAR in conducting technical assessment on proposed sites for central hatcheries and development of detailed engineering design and production plans.

References

Websites

International Disasters Database UC Louvain, Belgium www.emdat.be

Philippine Statistics Authority (PSA) OpenStat http://openstat.psa.gov.ph/

DA-BFAR internal and institutional documents

Bangus Development Plan 2020-2024, RD. Wilfredo Cruz, National Focal Person

Tilapia Commodity Program, 2018, Ma. Judecel Danting, National Focal Person

300 Fish cage Project, 2018. Roy C. Ortega, DA-BFAR Aquaculture Division

Philippine Fisheries Profile, 2016

Comprehensive National Fisheries Industry Development Plan (CNFIDP), 2008.

Potential Areas for Milkfish Marine Cage Operations, 2019.

Other references

Asian Development Bank. 2005. An Impact Evaluation of Genetically Improved Farmed Tilapia and Their Dissemination in Selected Countries. ADB, Pasig City, Philippines

Asian Development Bank. 2007. Strategy for Sustainable Aquaculture Development for Poverty Reduction (ADTA: PHI 4708), Philippines., Pasig City,

DA-BFAR and DOST-PAGASA. 2017. Impact Management of Weather Systems in Aquaculture. DA-Bureau of Fisheries and Aquatic Resources and DOST- Philippine Atmospheric, Geophysical and Astronomical Services Administration. PDF versions available upon request. Roy C. Ortega, National Project Coordinator (kaulayao@yahoo.com)

DOST-WorldFish. 2016. Aquaculture Futures: Fish Supply and Demand Scenarios and the Sustainable Growth of Aquaculture in the Philippines. DOST-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development Council and WorldFish-Philippine Country Office, 2016, Los Banos, Laguna

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO

FAO Fisheries and Aquaculture Technical Paper 614 (2018) “Better management practices for feed production and management of Nile tilapia and milkfish in the Philippines”;

FAO Fisheries and Aquaculture Technical Paper 583 (2013). “On-farm feeding and feed management in aquaculture”

Ortega, R.C. 2013. The emergence of the cheapest farmed freshwater food fish in the Philippines”. International Symposium on Small-scale Aquaculture Extension. Dec. 2-6, 2013. UN FAO Regional Office and Network of Aquaculture Centers in Asia and Pacific, Bangkok, Thailand.

PSA. 2017. Consumption of Selected Agricultural Commodities in the Philippines Vol. 2, Philippine Statistics Office, Quezon City, Philippines

R.A. 7161 Of 1991 “An Act Incorporating Certain Sections of the National Internal Revenue Code Of 1977, As Amended, to Presidential Decree No. 705, As Amended, Otherwise Known as “The Revised Forestry Code of The Philippines,” and Providing Amendments Thereto by Increasing the Forest Charges on Timber and other Forest Products”

R.A. 10654. An Act to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing, Amending Republic Act No. 8550, Otherwise Known as "The Philippine Fisheries Code of 1998," and For Other Purposes

R.A. 7586. An Act Providing for the Establishment and Management of National Integrated Protected Areas System, Defining Its Scope and Coverage, and for Other Purposes

REPUBLIC ACT No. 11038. An Act Declaring Protected Areas and Providing for Their Management, Amending for This Purpose Republic Act No. 7586, Otherwise Known as the "National Integrated Protected Areas System (NIPAS) Act of 1992" and for Other Purposes

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Report on Sustainable Aquaculture and Resource Enhancement in Thailand

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Abstract

Aquaculture in Thailand has continuously developed to become an important aquaculture producer in the world. This is possible because of Thailand's geographical location which is suitable for aquaculture, climate, and experienced farmers. The types of aquaculture in Thailand consist of natural, semi-intensive, intensive and super-intensive farming. The main freshwater aquatic species cultured in Thailand are Nile tilapia (*Oreochromis niloticus*), hybrid catfish (*Clarias macrocephalus x C. gariepinus*), and giant river prawn (*Macrobrachium rosenbergii*); Pacific white shrimp (*Litopenaeus vannamei*), giant tiger shrimp (*Penaeus monodon*), Asian sea bass (*Lates calcarifer*) and grouper (*Epinephelus* sp), in brackish water. Yield from marine shrimp (*Litopenaeus vannamei*, *Peneaus monodon*) culture comprised 64 % of the total marine aquaculture production. Shrimp aquaculture, a development of the agricultural industry, is income generating and creates jobs in various parts of the country. The rapid growth in shrimp production resulted in problems with cumulative impacts; one of the issues is the occurrence of epidemic diseases. Diseases have emerged as a major constraint to the sustainable growth of the shrimp aquaculture industry. Shrimp diseases have caused significant losses in production and jobs, reduced earning, export restrictions, failure and closing of business and decreased the confidence of consumers. Aquaculture, in this digital period of technology is mainly used in the aquaculture of marine shrimp — development of feeding applications, notification of aquatic animal diseases, proper aquaculture management. Technology is also used in the aquatic animal health examination to get accurate information to timely solve underlying problem (s); and thus reduces severe damage to the crop.

The new fisheries law and legal policies in Thailand recognize the importance of the aquaculture industry. The new order has a clear direction to prevent aquatic animal diseases from importing controlled aquatic animals, including the standard law for Pacific white shrimp hatchery including promoting environmentally friendly practices. The present Department of Fisheries (DOF), Thailand has encouraged standard farm to shrimp farmer including Good Aquaculture Practice (GAP), Code of Conduct (CoC), Thai Agriculture Standard (TAS): 7401, 7422, 7432 and 9000, Compartment (Biosecurity system) and Organic standard farm. The DOF has promoted aquaculture to achieve sustainability and enhancement of fishery resources, Therefore developed monitoring system, controlling system, surveillance system, and traceability system for aquaculture. To encourage farmers to have environmentally friendly aquaculture and products safe for consumers.

Problems and Challenges of Aquaculture in Japan

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Note: parts of contents of this report have been published elsewhere

Abstract

This paper describes the present status of aquaculture in Japan. The production volumes of many of the wide variety of aquatic organisms cultured in Japan, including finfishes, bivalves, crustaceans and seaweeds, are on a continuing decreasing trend. According to the national statistical data published by the Ministry of Agriculture, Forestry and Fisheries, the total marine aquaculture production decreased by 20 % by volume in 20 years since 1996 (1.3 million t). In fed aquaculture of marine finfishes, productions of the red seabream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*) and horse mackerel (*Trachurus japonicus*) have declined markedly in both volume and value. On the other hand, productions of Pacific bluefin tuna (*Thunnus orientalis*), which has been included in the statistics since 2012, is increasing remarkably because of the strong affinity of Japanese consumers and the dwindling wild population. Production of white trevally (*Pseudocaranx dentex*), which is recognized as a luxury foodstuff, is also increasing. Productions of yellowtails (*Seriola* spp.) and pufferfish (*Tetradontidae* spp.) are rather stable in terms of volume and value. International demand for yellowtails is growing, and the export is expanding. As for unfed culture of bivalves, productions of oysters (*Crassostrea* spp.) and Japanese scallop (*Mizuhopecten yessoensis*) were severely impacted by the Great East Japan Earthquake in 2011, and the production of the oysters, which is on a long-term decreasing trend, has not recovered to the level prior to the earthquake. Production of the major seaweeds (*Pyropia yezoensis*, *P. tenera*, *Saccharina japonica*, *S. angustata* and *Undaria pinntifida*) are all on a continuous decreasing trend both by volume and value. Inland (freshwater) aquaculture production is only about 3 % of the marine aquaculture production by volume. About 70 % of the freshwater aquaculture production value comes from Japanese eel (*Anguilla japonica*), which has been increasing since 2002 due to the increasing unit price despite the decreasing production volume. The insufficient supply of wild glass eel is a problem for the eel aquaculture.

The reduced aquaculture production is partially due to socio-economic and environmental reasons. For fed aquaculture, increasing feed cost and international competition are the major issues. Diseases also remain to be a problem. Prevalence of vaccination has reduced the disease damage from about 10 to 4 % of the total production value since around 2000, but the emergence of new diseases continues to occur. Infectious diseases have long been a problem in kuruma prawn (*Marsupenaeus japonicus*) aquaculture. Red tides still occur almost every year, causing damages mostly in western Japan.

For unfed aquaculture, the reduced production is considered to be related with oligotrophication of coastal waters. Intensive reduction of terrestrial nutrient loads by advances in wastewater treatment is thought to have reduced the seaweed productivity, as well as other primary production, resulting in reduced productivity of not only unfed aquaculture of bivalves but coastal fisheries in general. Social factors are also involved. Production declines of many of these species are partially attributed to the reduced labor force due to aging and insufficient recruitment of farmers. The number of management body of oyster aquaculture, for example, decreased from 4,349 in 1963 to 2,018 in 2013. Structure of aquaculture industry in Japan (mostly privately-owned small business) and strong Japanese currency bring about the weak international market competitiveness.

Introduction

Fish and seafood have been the staple food for the Japanese since the ancient time. Japan has a long history of aquaculture, and many novel aquaculture techniques have been invented and developed (Tanigawa *et al*., 1966). A wide variety of aquatic organisms, both freshwater and marine, are cultured in Japan, including finfishes, bivalves, crustaceans and seaweeds. Some of the major cultured species are, for example, red seabream (*Pagrus major*), yellowtails (*Seriola quinqueradiata*, *S. dumerili* and *S. lalandi*), Japanese eel (*Anguilla japonica*), Pacific oyster (*Crassostrea gigas*), Japanese scallop (*Mizuhopecten yessoensis*), kuruma prawn (*Marsupenaeus japonicus*), Nori (*Pyropia yezoensis*) and kelps (*Saccharina japonica* and *S. angustata*).

In addition to these species, various other aquatic organisms are commercially cultured and consumed in Japan. However, despite the historically strong affinity of the Japanese to seafood and fish, aquaculture productions of many species are on a long-term decreasing trend. This paper describes the present status of aquaculture in Japan, and some of its problems and challenges being faced by Japan's aquaculture industry.

The aquaculture statistical data analyzed and presented in this paper are based on the national statistical data on the aquaculture production published by the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF). The statistical data on the aquaculture production of major species by volume and value from 1993 to 2016 for marine aquaculture and from 1998 to 2016 for inland aquaculture are available online (http://www.maff.go.jp/j/tokei/kouhyou/kaimen_gyosei/index.html). Statistical data of the Fisheries Census are available online in e-Stat website (https://www.e-stat.go.jp/en). The statistical data are made available by the Ministry of Internal Affairs and Communications to reveal the situation surrounding the fisheries industry in Japan, such as the production and employment structures of fishing communities, and distribution and processing of fishery products. The data in the census include the number of fishery cooperatives, fisheries management entities, fisheries workers and fishing vessels.

Marine aquaculture production in Japan

Although the global aquaculture production is on an increasing trend (FAO, 2018), the total marine aquaculture production volume gradually decreased from 1.3 million MT in 1996 to 0.9 million MT in 2016 in Japan (19.6 % decrease over the past two decades. It dropped abruptly to 0.87 million MT in 2011 because of

tsunamis caused by the Great East Japan Earthquake. The tsunamis struck the coastlines of central to northern Japan and heavily devastated not only aquaculture facilities but also the coastal towns. The aquaculture production resumed the long-term decreasing track in 2012.

The total marine aquaculture production value continuously decreased and hit the bottom in 2011, and then it turned to an increasing trend after 2012). The production value was JPY 510 billion in 2016. This indicates the increase in unit price per production weight due to supply shortage and/or value addition efforts. On the other hand, seafood import is on a decreasing trend in Japan.

The total aquaculture production volume of finfishes is in a long-term decreasing trend, with a drop in 2011 due to the tsunamis (0.24 million MT and 0.23 million MT in 1996 and 2016, respectively). The tsunamis did not severely devastate the production of finishes except for Coho salmon (*Oncorhynchus kisutch*), the production level of which decreased from 15 thousand t in 2010 to 116 t in 2011. Coho salmon aquaculture is conducted mostly along the northeast pacific coast close to the epicenter. The production increased to 9.7 thousand t in 2012.

Yellowtails (i.e. the statistics is pooled for *S. quinqueradiata*, *S. dumerili and S. lalandi*) and red seabream are the two major marine finfish species cultured in Japan (0.13 million MT and .06 million MT, respectively in 2016). The aquaculture productions of these two species were not severely damaged by the tsunamis. Aquaculture production of the yellowtails is rather stable, and approximately 12% of the production is exported overseas in recent years, to the U.S.A. being the largest importer. Meanwhile, the production of red seabream was on a long-term decreasing trend before the tsunamis, and it turned into an increasing trend after 2014.

The aquaculture production value of the finfishes decreased from 1997 to 2005 (JPY 190 billion). It then turned into an increasing trend reaching JPY 240 billion in 2016. The recent increase in production value is partially due to the new inclusion of Pacific bluefin tuna (*T. orientalis*) aquaculture to the statistics in 2012.

The aquaculture production of marine molluscan shellfish (mostly oysters and scallops) was relatively constant at around 0.45 million MT before the earthquake in 2011. The earthquake devastated the production in 2011 (0.26 million MT), and did not recover until 2016 (0.37 million MT). In contrast, the production of the molluscs grew by 1.7 times in value from 2012 (JPY 57 billion) to 2016 (JPY 99 billion. Japanese scallop production was around 0.23 million MT before it went down to 0.12 million MT in 2011. It recovered to 0.25 million MT in 2015, exceeding the level prior to the tsunamis. The production value of the scallop showed a jump after 2012 (JPY 26 billion), reaching JPY 62 billion in 2016. While the oyster production volume was gradually decreasing before the earthquake (0.2 million MT in 2010), it persisted even after the earthquake (0.16 million MT in 2016). The production value of oyster aquaculture increased after the earthquake to a lesser extent as compared to that of the scallop (JPY 350 billion in 2016).

Global aquaculture production of seaweeds has more than doubled since 1995 (0.3 million MT in 2016). The rapid growth in the aquaculture of tropical species (*Kappaphycus alvarezii* and *Eucheuma* spp.) in Indonesia as raw material for carrageenan extraction has been the major contributor to growth in seaweed production in the recent past

(FAO, 2018). Seaweed aquaculture is primarily directed towards direct human consumption in Japan, and they are as important as finfishes and crustaceans. Nori (*Pyropia* spp.) for example is the second-largest aquaculture product (JPY 1000 billion in 2016) following yellowtail (JPY 1200 billion) in Japan. The aquaculture production of nori is on a decreasing trend regardless of the high demand in Japanese market over the past two decades. However, the aquaculture production of seaweeds is in a long-term decreasing trend. The production volume and value of seaweeds in 2016 (0.39 million MT and JPY 1200 billion) were 26 % and 12 % less than those in 1993, respectively.

Aquaculture of kelps (*Saccharina* spp,) and wakame (*Undaria* spp.) are operated mainly in Tohoku and Hokkaido regions and were severely damaged by the earthquake in 2011. Although the production of these seaweeds recovered rapidly in 2012, the long-term decreasing trend persists until now.

Inland aquaculture production in Japan

Freshwater aquaculture production is much smaller than that of the marine aquaculture in Japan. As of 2016, the former is only about 3 % and 20 % of the latter by volume and value, respectively. Japanese consumers tend to choose seafood in preference to freshwater fish, with Japanese eel as an only exception. Culturing freshwater molluscs, crustaceans and algae is not at all that common in Japan. Globally available freshwater aquaculture species, such as common carp (*Cyprinus carpio*, 3100 t in 2016) and tilapias (statistical data available only until 2000, 434 t) are also produced in Japan, but they are not consumed in large quantities.

The production volume of freshwater finfishes, including Japanese eel, has been on a continuous decreasing trend. It decreased by 42 % between 1998 and 2018 (0.032 million MT). The production of major species, such as common carp, ayu (*Plecoglossus altivelis*) and rainbow trout (*Oncorhynchus mykiss*) are all decreasing.

Meanwhile, the production value decreased from 1993 (JPY 920 billion) to 2002 (JPY 440 billion). From then, it has subsequently been on an increasing trend, mostly owing to the increased production value of Japanese eel. The production value in 2016 exceeded that of 1993 (JPY 940 billion). Japanese eel is by far the largest freshwater aquaculture product, accounting for 70 % of the total freshwater aquaculture production value. Wild caught glass eel is used as seeds for the eel aquaculture, and the availability of the seeds is diminishing. Japanese eel has been listed as endangered species in the IUCN Red List of Threatened Species. The soaring price of the glass eel is a problem in the recent past (JPY 3.0 million/kg in 2018, Fisheries Agency, 2019). Studies on artificial spawning of Japanese eel were started in 1960s, and the National Research Institute of Aquaculture finally succeeded in the full life cycle culture of Japanese eel in laboratory scale in 2010. However, there are still methodological problems that need to be solved in leveling up the seed production to full industrial scale.

Causes of the declining aquaculture production in Japan

Aquaculture production of many marine and freshwater species in different taxa is declining by volume in Japan for the past twenty years. Great East Japan Earthquake and consecutive tsunamis devastated the production of many species in 2011.

However, the long-term decreasing trend through 2011 and thereafter is not attributed to the earthquake. Although, the production value of some species is increasing, aquaculture activities as a whole are considered to be declining in Japan.

The reduced aquaculture production is due to socio-economic and environmental reasons. For fed aquaculture of finfishes and shrimps, soaring feed cost (Demura, 2010) and international competition are some of the major issues. Surge in the price of fishmeal, which is the main ingredient of compound feed for aquaculture, is putting pressure on the profits of aquaculture business worldwide, and Japan is not an exception since fish meal is mostly imported in Japan. The use of plant protein alternative to fishmeal is still limited.

Diseases also remain to be a problem in aquaculture. In marine fish aquaculture, such diseases as streptococcosis and pseudotuberculosis became widespread in the past. Today, vaccines for major diseases have become available, and the occurrence of fish diseases, as well as the use of antibiotics decreased dramatically (Fisheries Agency, 2014). Prevalence of vaccination has reduced the disease damage from about 10 to 4 % of the total production value since around 2000, but emergence of new diseases continues to occur. Infectious diseases, such as white spot disease, have long been a problem in kuruma prawn aquaculture. Invertebrates lack any form of immunological memory similar to that found in finfishes, making effective vaccination of invertebrates difficult.

With the implementation of the Total Pollutant Load Control System (TPLCS) to mitigate eutrophication of coastal waters in 1979, terrestrial nutrient (nitrogen, phosphorus and COD) load to enclosed sea areas (Tokyo Bay, Ise Bay and Seto Inland Sea) has been reduced. While the mitigation efforts have suppressed the occurrence of red tide drastically, red tides still occur sporadically almost every year, causing damages to fish and bivalve aquaculture mostly in western Japan.

For unfed aquaculture of bivalves and seaweeds (referred to as extractive species), the reduced production are considered to be related with the oligotrophication of coastal waters. Intensive reduction of terrestrial nutrient loads by advances in wastewater treatment under the TPLCS and other mitigation regulations have caused shortage of nutrient supply to algae, and it is thought to have reduced the seaweed productivity, as well as other primary production. This in turn has resulted in reduced productivity of not only unfed aquaculture of bivalves but coastal fisheries in general. Bleaching of nori thalli due to lack of nutrient supply has been a big problem in many parts of Japan, reducing the value of the products (i.e. darker color fetches higher price). “Suisan yousui kijun” (fisheries water standard, Japan Fisheries Resource Conservation Association, 2018) suggests the lower limit for total nitrogen and phosphorus be 0.2 mg/L and 0.02 mg/L, respectively, in coastal sea for sustainable primary production. Recently, some prefectural governments are experientially deregulating nutrient release from sewage treatment facilities in an attempt to improve coastal unfed aquaculture and fisheries productivities.

Social factors are also involved in the reduced aquaculture activities in Japan. Production declines are partially attributed to the reduced labor force due to aging and insufficient recruitment of farmers. The number of management body of oyster aquaculture, for example, decreased from 4,349 in 1963 to 2,018 in 2013. As of 2016, fishing industry employed 166,00 people, that was about half the number in 1993 (325,000 people). Decline of labor force is

not an issue particular to fisheries industry such that workers with a high education level being reluctant to be engaged in menial jobs. However, it is also related to the demographic challenges Japan is dealing with. Japan's fertility rate was 1.43 children per woman in 2017, and the population growth rate has been negative since 2011 (Ministry of Health, Labour and Welfare, 2018). The average age of the fishing industry employees was 56.7 years old in 2016.

The structure of aquaculture industry in Japan is mostly privately-owned small business, and it is economically less competitive than enterprises with a large capital. Traditional fishery systems including the fishery rights system and fishing license system hold back new entrants to aquaculture business. A Demarcated Fishery Right gives a fishery cooperative or an individual fisher the right to engage in coastal aquaculture in Japan, and it is traditionally hereditary. The Fishery Act and Fishery Cooperative Act were drastically revised for the first time in 70 years in 2018 to promote fisheries and aquaculture industry. The revision includes transparency of licensing process of fishery rights.

References

Demura, M. 2010. gyofun kakaku no doukou to yoshokugyogyou heno eikyo (trends in fish meal price and its effects on aquaculture). Norinkinyu 2010-10: 45-49 (in Japanese).

e-Stat. https://www.e-stat.go.jp/en

Fisheries Agency. 2014. FY2013 Trends in Fisheries FY2014 Fishery Policy, White Paper on Fisheries: Summary Pp38.

Fisheries Agency. 2019. A handout of the 95th meeting of the working group on fisheries resource management, fisheries policy council (in Japanese) http://www.jfa.maff.go.jp/j/council/seisaku/kanri/attach/pdf/190605-20.pdf

IUCN Red List of Threatened Species (Japanese eel, *Anguilla japonica*) https://www.iucnredlist.org/species/166184/1117791)

Japan Fisheries Resource Conservation Association (2018) Suisan Yousui Kijun 8th edition.

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. License: CC BY-NC-SA 3.0 IGO

Ministry of Agriculture, Forestry and Fisheries of Japan. kaimen gyogyou seisan toukei chousa (statistical survey on open water fisheries production) http://www.maff.go.jp/j/tokei/kouhyou/kaimen_gyosei/index.html

Ministry of Health, Labour and Welfare (2018) Annual Report on Health, Labour and Welfare 2017. Pp. 508

Tanigawa, E., Tamura T., Kanamori, M., Arai, D. 1966. Suisan-gaku-tsuron (complete fisheries science). Koseishakoseikaku, Tokyo, Pp. 306 (in Japanese).

Resource Enhancement: concepts, learnings, and future directions

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Abstract

Fish and fishery products are always in high demand causing pressure on world fish supply. The world's wild capture fisheries resources ultimately reached its peak around the early 90s and plateaued at around 90 million tonnes. The stagnancy in wild fisheries production poses an alarm to the ever-growing human population. Fortunately, at around the same time, aquaculture production has filled some gap in seafood supply with over 100 million tonnes produced annually. However, there are various concerns about aquaculture and its sustainability. This is where the idea of aquatic resource enhancement comes in, not only to increase fish yield for food but also to compensate for losses caused by anthropogenic interventions, while promoting environmental rehabilitation and conservation. Resource enhancement, as a whole, can include various concepts on sustainable development, habitat conservation and improvement, ecological management, and aquaculture-based stock enhancement. This paper highlights the development of some resource enhancement programs worldwide and provide some examples particularly those from the Southeast Asian Region. We will attempt to tackle some successes and failures, as well as review past and recent experiences to extract important learnings. Based on these lessons, future directions of how resource enhancement initiatives can be made more efficient and sustainable. As a general rule, we recommend that in order to increase chances of success for programs on resource enhancement, it has to be science-based, there needs to be inclusive and participatory planning and management involving all stakeholders and adheres to responsible culture practices. Moreover, there should be concurrent efforts in reducing fishing pressures, as well as in protecting and rehabilitating natural ecosystems.

Introduction

Seafood and seafood products are among the top sought-after and expensive food commodities. Because of the apparent abundance of seafood in commercial markets worldwide, it is thought that fish supply can be limitless. The increasing demand for seafood causes more pressure on wild fish supply, especially with the continuous increase in global population that is expected to reach over 8 billion by 2020 (UN, 2019). However, global capture fishery has been recorded to remain at around 90 million metric tons (mt) per year for over two decades now

(**Figure 1**). Some evidence of overfishing is shown to be associated with smaller-sized fish composition in catch, as well as decreasing size-at-maturity of wild fish species (Hunter *et al*., 2015). According to FAO, IUU or illegal, unreported, and unregulated fishing is one of the biggest threats to marine ecosystems that weaken the crucial efforts in sustainably managing fisheries resources while conserving marine biodiversity (FAO, 2016). The sustainability of sea-sourced resources and ecosystems are also more seriously being considered in these modern times. The United Nations established 17 Sustainable Development Goals (SDGs) in 2015, within which SDG14 specifically focuses on "Life Below Water" which aims to sustainably manage and protect marine and coastal resources and ecosystems (UN, 2015).

Aquaculture has been providing volumes of fish and aquatic products since the eighties (**Figure 1**), contributing >50 % of global seafood consumption. However, environmental issues and problems with aquaculture have been raised, pinpointing the negative effects of conversions of mangrove areas to fish ponds (Primavera, 2005), pollution from wastes and effluents (Primavera *et. al*., 2007), and even direct effects on reducing wild fish populations (Naylor *et. al*., 2000). On the bright side, research and development efforts have improved modern aquaculture. Research thrusts are not only in meeting production targets, but also consider health, biosecurity, better-sourced feeds, and environmentally-friendly culture systems. Most of these are being addressed by research institutions like the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD).

Ultimately however, the question is not only on whether or not we will have enough fish to eat in the future, but also whether natural aquatic resources and coastal ecosystems will still be in good condition. This is where the idea of resource enhancement is based upon.

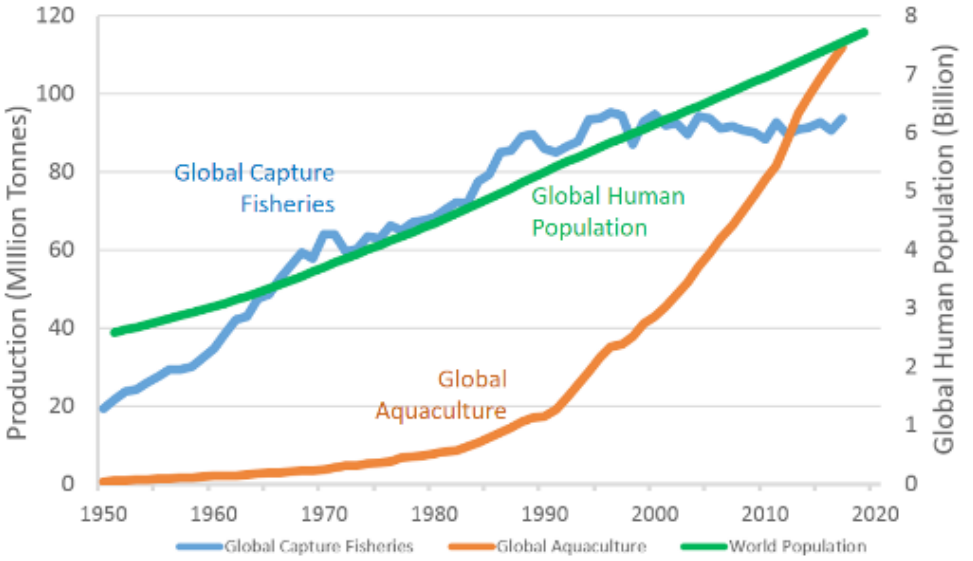

Figure 1. Global trends in capture fisheries and aquaculture (data from FAO, 2019) and world population (data from UN, 2019)

Resource enhancement: concepts, objectives and strategies

Resource enhancement has been called and defined in various ways. Terms like stock enhancement (Harada and Matsumiya, 1992; Ziemann, 2001; Drawbridge, 2002; Bell *et al*., 2006), restocking (Bannister, 1991; Bell *et al*., 2006), sea ranching (Blaxter, 1994; Bartley, 1999; Arnason, 2001; Drawbridge, 2002), coastal mitigation (Cowx, 1994; Bartley, 1999; Radtke and Davis, 2000), augmentation (Bannister, 1991; Cowx, 1994; Bartley, 1999), and addition (Bannister, 1991; Rowland, 1994) have been used interchangeably in the past because of similarity in the general goal of increasing fishery stocks in the sea.

Early stock enhancement initiatives dated back to 1867 with shads (*Alosa sapidissima*) in New England (Stickney, 1996) and chum salmon (*Oncorrhynchus keta*) in Japan in 1876 (Oshima, 1993). In 1884–1985, USA and Norway also initiated stock enhancement of finfishes (Liao, 1999). The first salmon hatchery in the United States was established in the 1870s (Blankenship and Leber, 1995) and the release of marine finfish followed, including the Atlantic salmon, flounder, haddock, cod and pollock (Blaxter, 2000; Leber, 2004). Meanwhile, the earliest record of stock enhancement activities in Europe was the release of plaice in Norway and Scotland in 1882 and 1894, respectively and transplants of the same species in the Baltic and North Seas in 1893 (Blaxter, 2000). Since then, many other attempts and programs for stock enhancement followed.

Stock enhancement of invertebrates like mollusks also started as early as 1900s for scallops in Norway, but was more commercially implemented starting 1970 in Europe and Japan (Salvanes, 2001). Specifically, scallops *Patinopecten yessoensis* were produced for release in Japanese waters (Kitada, 1999). Other species released, including some echinoderms, were queen conch *Strombus gigas* (Ray *et. al*., 1994), topshell *Trochus niloticus* (Crowe *et. al*., 2012), abalone *Haliotis assinina* (Salayo *et. al*., 2020), and sea cucumber *Apostichopus japonicus* (Yokoyama, 2013) in Japan and *Holothuria scabra* (Purcell *et. al*., 2012) in the West Pacific. In China, sea ranching of abalone, scallops and sea cucumbers started in the 1980s (Jia and Chen, 2001). Other than as food resource, endangered species were also reseeded for conservation purposes like the giant clams *Tridacna* spp. (Mingoa-Licuanan and Gomez, 2002) and sea horses *Hippocampus* spp. (Okuzawa *et. al*., 2009).

Crustacean stock enhancement programs also started early, like the release of newly-hatched eggs and newly-settled juveniles of the European lobster *Homarus gammarus* in southern Norway in 1889 (Salvanes, 2001). Crabs also had extensive release studies such as the blue crabs *Callinectes sapidus* (Zmora *et. al*., 2005), *Portunus trituberculatus* (Okamoto, 2004), and mangrove crab Scylla species (Le Vay *et. al*., 2008). Shrimp stock enhancement also started since the 1960s in Japan with *Penaeus japonicus* (Kitada, 1999; Fushimi, 2016), and since 1980s in China with *Penaeus chinensis* (Wang, 2006). Unlike other species, however, crustacean stock enhancement programs were relatively few because of various problems in monitoring brought about by tagging difficulty due to molting (Altamirano and Kurokura, 2010).

In general, the release of hatchery-produced juveniles to the wild for whatever objective has been referred to as stock enhancement. However, Bell *et. al*. (2008) proposed only three main categories and definition of marine resources enhancement. “Stock

enhancement" is defined as the release of hatchery-produced juveniles to augment natural supply and optimize harvests by overcoming recruitment limitation. This is viewed to have a more public oriented benefit, where the stocks will become part of the common resources that anybody can eventually harvest. "Restocking" is the release of cultured juveniles into wild populations to restore severely depleted spawning biomass and has an environmental-oriented benefit. "Sea ranching" also make use of hatchery-produced juveniles released into unenclosed marine and estuarine environments for harvest at a larger size through a "put, grow, and take" operation. Unlike the former two categories, sea ranching is mainly for private benefit, where those stocks are associated to a defined area, and secured and "cultured" by known operators. Although spill over ecological and public benefits can also be derived from sea ranching (e.g. Juinio-Meñez *et al*. 2013).

In recent decade, programs on stock enhancement, restocking and sea ranching have become more holistic. In many cases, these do not just focus on increasing the target "stocks", but largely encompass concerns of ecological sustainability, as well as, of human's capacity development and socio-economic welfare. In this paper, therefore, we will be referring to the term "Resource Enhancement" that incorporates the general stock enhancement concepts mentioned above of increasing fish and shellfish yield for food, recreation or conservation, but also includes programs for compensating losses caused by overfishing and environmental degradation by promoting resources and habitat conservation, as well as the capacity enhancement of human resources.

Resource enhancement programs vary in objectives and reasons for implementation. Cowx (1994) defined four main reasons for stocking, namely:

(1) mitigation – a voluntary stocking exercise as a fishery protection scheme after some environmental perturbation like dam construction, land drainage works, etc.;

(2) enhancement – a principal method used to maintain or improve stocks where production is actually, or perceived to be, less than the water body could potentially sustain, but where reasons for the poor stocks cannot be identified;

(3) restoration – carried out after a limiting factor to stock recovery or improvement has been removed or reduced, like water quality improvement or habitat restoration; and

(4) introduction – attempts to establish a new stock that was not previously present because of natural barriers or evolutionary isolation, or where new exotic species are introduced into existing fisheries in an attempt to increase diversity or improve yield.

In Southeast Asia, Lebata-Ramos and Doyola-Solis (2016) summarized the main reasons for conducting resources enhancement which are:

(1) to increase in production of target species;

(2) to increase food supply and income;

(3) to revive endemic species;

(4) to conserve endangered species;

(5) to rehabilitate degraded natural habitats;

(6) to restore spawning and feeding grounds;

(7) to enhance fish sanctuaries;

(8) to act as biological control;

(9) to promote aquaculture; and

(10) to develop recreational fishing.

In some cases, however, a resource enhancement program may address a multiple of these objectives.

Alongside these objectives, approaches and strategies for resource enhancement programs also vary. Welcomme and Bartley (1998) mentioned that there are two main strategies for management and enhancement of water resources, and these can be differentiated between strategies implemented in "developed" and "developing" countries (**Table 1**). Stocking strategies in "developed" countries are geared towards ecological goals, particularly the enhancement of recreational fisheries and protection of species diversity. In fact, the preference for sports fishing which are predominantly in developed countries, has led to early adoption of enhancement techniques and these were usually coupled with some form of habitat maintenance and conservation (Welcomme and Bartley, 1998). These programs are also heavily subsidized by the government like in the cases of Japan (Kitada, 1999) and the USA (Leber, 2004). Government hatcheries are capital intensive and the associated aquaculture techniques are also intensive in design, industrialized, and employ latest technology and gearing towards higher returns and profit. On the other hand, such programs in "developing" countries are mostly economically-driven, specifically for food security and income generation which also involve some form of habitat modification and intensive broadcast of seeds (Welcomme and Bartley, 1998). Aquaculture efforts to support resource enhancement in developing countries are extensive but labor-intensive, rural in nature, and lack enough funding for infrastructure development despite adequate physical potential of the area.

Table 1. Differing strategies for resource enhancement programs between developed and developing countries (after Welcomme and Bartley, 1998)

	Developed Countries	Developing Countries
Objectives	Conservation	Provision of food
	Recreation	Income generation
Mechanisms	Sport fisheries	Food fisheries
	Habitat restoration	Habitat modifications
	Environmentally-sound stocking	Intensive restocking
Economic	Intensive, modern aquaculture	Extensive, rural aquaculture
	Capital-intensive	Labor-intensive
	Profit	Production

Some resource enhancement initiatives

Volume and number of species

As a positive fisheries management tool and to boost fishing production, scientists and fisheries managers have been looking at ways of enhancing fish stocks for more than a century (Liao, 1999; Blaxter, 2000). Since then, well over than 300 species were estimated to have been used for release worldwide (Welcomme and Bartley, 1998), and >290+ of these are freshwater (Welcomme, 1992), signifying that marine restocking is relatively uncommon (Brown and Day, 2002). Similarly, there are more species released in freshwater bodies than in the sea of various countries in Southeast Asia. Freshwater species like the Nile tilapia (*Oreochromis niloticus*) has been the major commodity for release in small waterbodies in Savannakhet Province in Laos PDR since 1994, although the government has been recently promoting the enhancement of the Indian major carps (*Cirrhinus mrigala* and *Labeo rohita*) as well (Garaway *et. al*., 2006). In the whole Southeast Asia, the most common are barbs, particularly the silver barb *Barbonymus gonionotus*, and tilapia *Oreochromis niloticus* with Malaysia and Thailand leading the most number of freshwater species targeted for enhancement (see summary table in Lebata-Ramos and Doyola-Solis, 2016). On the other hand, the Philippines ranked first for the most number of marine species released for resource enhancement, including various species of high value finfishes, crustacean, mollusks and echinoderms. In comparison, about 80 species are being ranched or researched for eventual stocking in Japan alone (Fushimi, 2001; Salvanes, 2001).

A recent systematic review of the global marine stock enhancement reported a total of 187 species comprised mainly of marine fish, salmon, crustaceans, mollusks and other species was released by 20 countries between 2011 and 2016 (Kitada, 2018). Japan released the largest number of species (72) followed by Taiwan (24 marine fish species only), USA (22 species), China and South Korea (14 species) and Australia (seven species). Other countries released only one or two species in considerable quantities. Salmon hatchery release is the largest release program in the world. Five countries (USA, Canada, Russia, South Korea and Japan) released seven species of salmon in the millions. The US alone was reported to release 2 billion juveniles in 2016 (Kitada, 2018).

Recovery and enhanced catch

Although stock enhancement activities have been done more than a century ago, the approach during those early times can be categorized as being focused on "production" where the main concern was achieving higher release volume and magnitude (Leber, 1999). Especially for developing countries, the anticipated increase in stock volume is viewed to have direct impact in terms of increasing sales from catch. A specific example for this benefit is shown by the stock enhancement activities of sea bream *Pagrus major* in Japan (Fujii, 2016). Sequential releases of red sea bream stocks since 1983, resulted in detectable increase of harvests since 1990, and even recorded abundance of wild recruits since year 2000 (see Fujii, 2016; Kitada *et. al*., 2019). In another species, the Japanese flounder *Paralichthys olivaceus* stock enhancement in Fukushima Prefecture, Japan has achieved a 30% recapture rate, and the cost-benefit ratio is estimated to be more than 300% (Masuda and Tsukamoto, 1998). Through purely theoretical calculations, Yulianto *et al*., (2019) estimated the potential benefit of stock enhancement of grouper *Epinephelus fuscoguttatus* in Karimunjawa Islands,

Indonesia to be 1.27–1.69 USD (based on 1000 released fish, 10–15 cm size), but can be highly variable depending on weather conditions.

In terms of general recovery, a recent review by Kitada (2018) from 37 studies covering 24 species, revealed that the overall weighted mean recovery rate was only at 8.1 ± 8 %. In general, recovery rates for fish species (e.g. cod, sea bream, halibut, mackerel) can be between 10–15 %, while crustacean species (e.g. shrimp, lobster, crabs) achieved a recovery rate of less than 6 %, with mangrove crabs at only 0.9 % (Kitada, 2018). Experimental releases of the tiger shrimps *Penaeus monodon* in Aklan, Philippines showed promising results whereby quantitative evaluation of recovery was recorded, even conservatively, at 8 % (Altamirano *et al*., 2016) (**Figure 2**). Shrimp releases in China since the 1980s had evaluated recovery of 0.001–1.88 % (Jia and Chen, 2001).

Unfortunately, the low recovery and negative economic returns for resource enhancement programs have been more common (Kitada, 2018). In Norway, releases of juvenile Atlantic cod (*Gadus morhua*) in 1980s and 1990s did not significantly increase cod production and catches (Svåsand *et. al*., 2000). Very high mortality of hatchery-bred juvenile topshell *Trochus niloticus* released in Australia, Indonesia and Vanuatu was recorded, which was mainly associated with predation (Crowe *et. al*., 2002). Mortality and high variation in recovery were also limited by the carrying capacity of the release sites, as in the case of red sea bream *Pagrus major* and the Japanese flounder *Paralichthys olivaceus* in Japan, whereby "cautious approach" to stock enhancement is recommended (Kitada and Kishino, 2006).

More than just enhancing catch

While there have been numerous efforts on resource enhancement, the major gap is in evaluating their impacts. Economic gain in terms of fishery production is often the intended target of large-scale efforts of long-term culture-based enhancements. However, smaller-scale community-based restocking and sea ranching efforts were also undertaken for conservation of species and at the same time development of supplemental

Figure 2. A local fisher (left) helps in monitoring the sizes of tiger shrimps caught daily, taking special note of tags (right) to indicate samples of released shrimps

sources of livelihood, as well as capacity building of local communities (Fushimi, 2016; Juinio-Meñez, 2016). Unintended impacts such as development of tourism attractions, ecotourism learning sites and community empowerment related to improve environmental/ecological conditions have also been realized in some cases particularly in Southeast Asia.

Efforts for the stock enhancement of the Japanese kuruma prawn *Penaeus japonicus* in Hamana Lake, Shizuoka, Japan was not quick and easy. It took some awareness molding among seven collaborating villages around the lake to realize some success in increased catch. Capacitated local communities were then able to implement shrimp releases and environmental monitoring by themselves since 1985 (Fushimi, 2016). In Thailand, capacity building and awareness campaign was implemented in Sriboya Island, Krabi Province, where various stakeholders of dog conch *Strombus canarium* were informed of its sustainable management and habitat conservation in 2013 (Manajit *et. al*., 2016). Through various public awareness activities, permanent dog conch conservation sites were established by local communities in the region where sustainable harvesting and management are now in place.

In the Philippines, the communal sea ranching of the sandfish *Holothuria scabra* was piloted in a small 5-ha area in Bolinao, Pangasinan as a means to help rebuild local population and provide a supplemental source of income to small fishers (Juinio-Meñez *et. al*., 2012). The sea ranch is managed by some members of a local small fishers' association (Samahan ng Malililit na Mangingisda ng Victory) and are responsible for guarding, maintenance and providing assistance in the monitoring. They share in the sales of harvested and processed sandfish from the sea ranch (**Figure 3**). A spawning population is maintained in the area because sandfish are not harvested until they reach >300 g, which is greater than the average size of sandfish that attain sexual maturity (>180 g) (Juinio-Meñez *et. al*., 2013). Increase in landed catch of sandfish is attributed by collectors to the efforts of the local managers in maintaining the sandfish sea ranch.

Restocking efforts for endangered species are also being carried out in the Philippines for giant clams whose natural populations have been depleted while the largest species, *Tridacna gigas*, was virtually extinct (Juinio *et. al*., 1989). Tens of thousands of cultured juveniles of the different species (*Tridacna gigas*, *T. derasa*, *T. squamosal*, *T. maxima*, *T. crocea*, *Hippopus hippopus*) have been produced in the hatchery, reared in the ocean nurseries, and restocked in over 40 locations throughout the Philippines (Gomez and Licuanan, 2006). Indication of natural recruitment from the restocked clams have been documented in at least two of these sites (Cabaitan and Conaco, 2017). Moreover, the projects also had

Figure 3. Local fishers participating in the communal sea ranching of sandfish in Bolinao, Pangasinan, Philippines

the unintended long-term impact of enhancing coastal ecotourism activities and revenues, where giant clam gardens have become part of tourist attractions in restocking sites that were well maintained, in partnership with the private sector and local government. Meanwhile, a theoretical study on the benefit of stock enhancement of grouper *Epinephelus fuscoguttatus* in Karimunjawa Islands, Indonesia, estimated a projected positive contribution to tourism around the islands by potentially generating 550,000 USD annually (Yulianto *et. al*., 2019).

Some problems and concerns

Stock enhancement studies were conducted extensively in many countries, mostly unscientifically, for more than a century but the success of these programs were apparently absent and unquantifiable (Welcomme and Bartley 1998; Cowx, 1999; Liao *et. al*., 2003; Bell *et al*., 2006). The long-lived constraint of stock enhancement or restocking activities is not only the lack of proof of success in terms of harvest and ultimate production, but also in terms of natural ecology, and effects on social dynamics of local fishing communities.

The case of abalone *Haliotis asinina* enhancement in Sagay, Negros Occidental, Philippines demonstrated the success of fishery recovery, but also highlights the role of aquaculture in mass-producing the seeds required for repopulating degraded target species and improve catch of beneficiary fishers (Salayo *et. al*., 2020). In many cases, however, release of stocks in the wild was done because of surplus in supply of hatchery-bred and reared individuals originally for aquaculture, like for shrimps in Japan and China (Fushimi, 1999; Wang, 2006). This has become a popular method of supplementing depleted stocks. However, the capability to produce abundant seeds from hatcheries and aquaculture farms is not enough reason to release juveniles and call it stock enhancement (Bell and Nash, 2004). Moreover, hatchery operation often only accounts for large quantities of seed production, rather than producing good quality and ecologically viable individuals (Fushimi, 2001; Brown and Day, 2002). Without careful considerations, these haphazard releases would eventually result in critical loss of stock fitness through ecological impacts and genetic introgressions (Kitada, 2018), negative interactions of released stocks with wild species and alter trophic dynamics (Cowx, 1994), possible transfer of diseases (Bartley *et. al*., 2006), and socio-cultural impacts on the local human communities (Garaway *et. al*., 2006; Altamirano *et. al*., 2015).

The limited number of broodstock, which were often spawned multiple times, to produce seeds for release may result in many anomalous genotypic and phenotypic traits. Kitada (2018) summarized the genetic risks of artificial propagation that may include:

(1) loss of genetic diversity;

(2) loss of fitness and performance;

(3) change in population composition,; and

(4) change in population structure.

This is why, as a precautionary approach, it is recommended to use only local stocks of wild broodstock to maintain genetic integrity of local populations, as in the case of the sandfish *H. scabra* that showed highly defined regional genetic structures in the Philippines (Ravago-Gotanco and Kim, 2019).

Problems with the local fishing communities can also arise because fisheries management programs, including stock enhancement, entail some kind of social modification like fishing limitations (Altamirano *et al*., 2015). Often, the success of the resource enhancement program can be largely dependent on the presence of a strong local community leadership, and that the community members have direct access to the benefits while being agreeable and able to adapt the technology required (Garaway *et al*., 2006). There is also that inherent danger of dependency by the benefiting community on the stock enhancement activity. Lenanton *et al*., (1999) reported that should the artificial releases be successful in increasing catch and income, there might be the desire to continue such activity over longer periods, while creating the tendency to overlook the original subtle causes of the decline.

In this case, the local communities will continue to rely on this artificial and virtual solution, rather than pursuing the ultimate goal of having naturally sustainable aquatic resources. Therefore, stock enhancement or sea ranching should not be seen as a substitute for the long-term conservation and management of valuable aquatic resources (Liao et al., 2003). Blankenship and Leber (1995) summarized that to effectively recondition depleted stocks, fishing effort must first be regulated; second, degraded nursery and spawning habitats must be restored; and third, only then can stocks be replenished through stock enhancement or restocking.

Future considerations for responsible resource enhancement

Science-based and systematic

The common approach during the early times of stock enhancement can be categorized as being focused on "production" where the main concern was achieving higher release volume and magnitude (Leber, 1999). Moreover, the advent of stock enhancement activities was started with the simplistic idea that production could be increased by releasing eggs or larvae of a certain species into coastal or marine waters (Welcomme and Bartley, 1998). The period governed by this simplistic notion was branded as the "denial phase" by fisheries scientists and it is only in the late 90s, where the "science" of stock enhancement began when critical thinking emerged and scientific objectives and hypotheses were formulated (Leber, 1999).

Whatever the purposes and strategies defined for stock enhancement, it is most important to consider various scientific aspects to ensure the applicability and feasibility of such programs in achieving those goals. In contrast with the way release programs were conducted in the past, modern stock enhancement activities need to be more scientific in approach. Given the long history of questionable stocking practices, and because of the rapidly expanding interest worldwide in starting new programs, it is essential to apply a substantial amount of science towards solving several key constraints to responsible application of stock enhancement technology (Blankenship and Leber, 1995). To develop an effective and sound stock-enhancement tool, the integration and coordination of research and expertise in several essential sub-disciplines of natural science and social science are imperative. For a successful resource enhancement program, the biology and ecology of target species must be thoroughly understood from production of seed stocks until monitoring and assessing the efficiency of release, as well as the conditions of the environment for release, carrying capacity of the

habitat, wild populations and diversity, factors that may contribute to mortality, and existing fisheries and social conditions (Fushimi, 2001; Liao *et al*., 2003; Garaway *et. al*., 2006).

One key in ensuring successful stock enhancement and restocking programs is following some pre-determined guidelines for systematic planning, implementation, monitoring and management. Blakenship and Leber (1995) have made a 10-point system for a responsible approach to developing, evaluating and managing marine stock enhancement programs. This system includes the following:

1. prioritize and select target species;
2. develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives;
3. define quantitative measures of success;
4. use genetic resource management to avoid deleterious genetic effects;
5. use disease and health management;
6. consider ecological, biological, and life-history patterns;
7. identify released hatchery fish and assess stocking effects;
8. use an empirical process for defining optimum release strategies;
9. identify economic and policy guidelines; and
10. use adaptive management.

Integrated, holistic, participatory and sustainable

Scientific studies on resource enhancement have been done in the recent years, but are only mostly aimed towards reducing cost of producing ecologically fit juveniles from hatcheries and to increase survival of released stocks, hoping that only these aspects can make stock enhancement or restocking viable (Howell *et. al*., 1999; Blaxter 2000; Bell *et al*., 2004; Leber *et. al*., 2004). However, current progress in research also involves various other concerns from basic biology and ecology of target species (Fushimi, 2001), health management (Bartley *et. al*., 2006), risk assessments, socio-economic studies, as well as social science perspectives (Garaway *et al*., 2006). Perhaps, equally important are the roles of fisheries managers, local governments, and especially the local fishing communities for effective and responsible stocking program (Liao, 2003; Garaway *et al*., 2006; Altamirano *et al*., 2015; Fushimi, 2016; Juinio-Meñez, 2016). Bell *et al*., (2006) highlighted that "restocking and stock enhancement programs are applied in complex human–environment systems, involving dynamic interactions between the resource, the technical intervention and the people who use it." In Laos, increases in catch of Nile tilapia in enhanced lakes was not realized immediately. The success was mostly dependent on the characteristics of the benefited community and on how they manage the resource available to them, making the resource users the most crucial factor in the determination of ultimate outcome of the resource enhancement programs (Garaway *et al*., 2006). Lorenzen *et al*., (2010) also emphasized the need for looking at the broader picture even at the earliest phase of the project development and defining about the purpose, identifying the players, and specifying alternative measures. This also means that all

players and stakeholders should actively participate, be scientifically informed, and therefore accountable for the planning, implementing and assessment of the potential contribution of enhancements.

The potential success of the enhancement program can be largely magnified when various phases and aspects of the production chain is considered. This was exemplified by the case of the scallop stock enhancement program in Japan where consideration was not only on ensuring the high quality of seedlings, but also in the improvement of the scallop general habitat, implementing proper farming management like crop rotation, and making sure that the marketing system was strong (Matsuda and Tsukamoto, 1998). It is also important to put in place some suitable and acceptable management interventions even before the release activities. Experiences in Laos for the enhancement of Nile tilapia exemplified that "the combination of access restrictions and stocking had a strong positive effect on total standing stocks" (Lorenzen *et al*., 1998). The authors also reported that releasing of stocks alone will not necessarily increase yields unless some "optimal management regimes can be identified and implemented by the management institutions."

The varying levels of success in stock enhancement programs around the world have resulted in various research concerns and scientific information generated has emphasized the importance of physiological, biological, morphological, and ecological attributes of the hatchery-reared juveniles (Fushimi, 2001) and their behavior, adaptations, and survival in the wild (Bell, 2005), as well as the social dimensions of the community (Garaway *et al*., 2006). All of these available information may already be harmonized to form part of policies and management arrangements, particularly in areas that were well-studied. At the present generation, it may be considered as a crucial crossroad for resource enhancement – from an "exploratory, research-oriented endeavor" to becoming a more useful set of tools in the "fisheries management tool box" (Lorenzen *et al*., 2013).

Culture and stock enhancement has to be incorporated within an integrated management framework that includes harvest regulation and protection of ecosystems and critical habitats. On a broader scale, a multi-criteria science-based approach can be used to delineate ecologically meaningful management units for resources as illustrated in the Philippines for sea cucumbers (**Figure 4**, UPMSI-DOST project). This includes understanding of biophysical and genetic connectivity, and distribution of habitats coupled with local governance capacity. The holistic framework for policy decision support can guide resource enhancement efforts to conserve genetic diversity and productivity in the long-term. In addition, priority areas for conservation and restocking has to take a wider ecosystem and trans-boundary approach to further ensure applicability and success (see example from Siriraksophon (2016) on regional trans-boundary refugia in Southeast Asia).

Conclusion

Resource enhancement is a century-old concept that has been ever evolving. Since its inception, some recurring themes of problems and concerns have become evident. Early stock enhancement programs

1. were focused on "production" and release volume which were considered to be not scientific;

2. made use of eggs and larvae which were eventually not measureable;

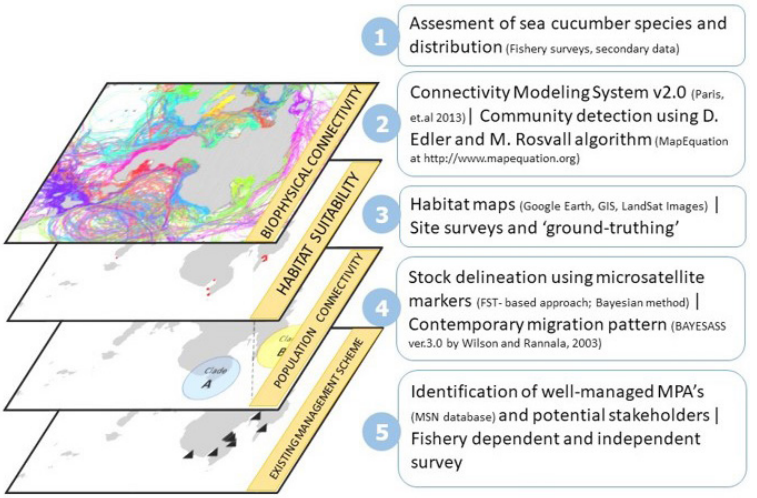

Figure 4. Integrated multidisciplinary approach to identify ecologically meaningful management units (UPMSI-DOST project)

3. used wild larvae or juveniles which may be considered to be not sustainable;

4. used of non-indigenous species which may not be ecologically-healthy;

5. lack coordination with other sector and not holistic; and

6. lack follow-through management and not integrated.

However, releases of juvenile fish and shellfish into freshwater or marine waters did increase seafood production to some extent, although monitoring were very well in place for most cases. Resource Enhancement has also evolved from doing haphazard releases, to being more holistic by incorporating habitat restoration and conservation. More and more has resource enhancement programs have engaged various stakeholders, especially the concerned local communities in developing countries, while promoting capacity building and awareness enhancement.

Although there are various and extensive experiences on resource enhancement demonstrated worldwide, whether for large commercial scale or for smaller experimental and community-based scale, resource enhancement will still need to evolve some more. The major challenges include:

(1) scaling-up production using scientific, holistic and responsible approach;

(2) sustaining production and engagement of partners while ensuring long-term ecological and socio-economic benefits;

(3) maintaining effective management and governance systems that are tailor-fit and acceptable to all stakeholders.

Resource enhancement programs need to be carefully planned. Bell *et al.* (2006)

emphasized that preliminary assessments should be carried out before investments on research and construction of facilities, and the need to have clear and specific objectives before any alternative management options are dismissed or delayed in lieu of stock enhancement. Additionally, Lorenzen (2005) promoted that studies on population dynamics and bioeconomic modeling in combination with participatory approaches in planning and implementation can provide a broad-based assessment of alternatives and help avoid unrealistic expectations and biased views and decisions.

Resource enhancement approach alone will not solve the problems of fisheries overexploitation. Programs should be integrated with other management approaches, that may already be in place (e.g. MPAs, effort control, etc.), and should be cognizant of long-term changes (e.g. effects of climate change), and should be aligned with sustainable development goals. Sustainable resource enhancement should be harmonized with responsible fishery practices and at broader scales to have real impact. For long-term sustainability, we reiterate that resource enhancement programs should be science-based, systematic, integrated, transdisciplinary, multi-sectoral, ecosystem-based and transboundary, participatory, responsible and sustainable.

If there is to be enough seafood in the future, resource enhancement must be practiced collaboratively and responsibly.

References

Altamirano, J.P. and Kurokura, H. 2010. Marking of tiger shrimp *Penaeus monodon* (Fabricius) juveniles: Comparison among inexpensive tagging options. La Mer 47(4): 33-45.

Altamirano, J.P., H. Kurokura, N. Salayo, D. Baticados, J.G. Suyo, S. Ishikawa. 2015. Community-based shrimp stock enhancement for coastal socio-ecological restoration in the Philippines. In: Romana-Eguia, M.R.R., F.D. Parado-Estepa, N.D. Salayo, & M.J.H. Lebata-Ramos (Eds.). Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species. SEAFDEC/AQD. pp 159-167.

Altamirano, J.P., Salayo, N., Kurokura, H., Fushimi, H., Ishikawa, S. 2016. Aquaculture-based restoration and stock enhancement of tiger shrimps in the Philippines. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp. 166-170.

Arnason, R. 2001. The economics of ocean ranching: experiences, outlook and theory. FAO Fisheries Technical Paper 413, 45pp.

Bannister, R.C.A. 1991. Stock Enhancement. ICES Mar. Sci. Symp. 192, 191-192.

Bartley, D.M. 1999. Marine ranching: a global perspective, In: Howell, B.R., Moksness, E., Svasand, T. (Eds.), Enhancement and Sea Ranching. Fishing News Books, Oxford, pp. 79-90.

Bartley, D.M., Bondad-Reantaso, M.G., Subasinghe, R.P. 2006. A risk analysis framework for aquatic animal health management in marine stock enhancement programmes. Fisheries Research 80, 28-36.

Bell, J.D., Bartley, D.M., Lorenzen, K., Loneragan, N.R. 2006. Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. Fisheries Research 80(1):1-8.

Bell, J.D., Leber, K.M., Blankenship, H.L., Loneragan, N.R., Masuda, R. 2008. A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. Reviews in Fisheries Science. 16, 1-9.

Bell, J.D., Nash, W.J. 2004. When should restocking and stock enhancement be used to manage sea cucumber fisheries? In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.F., Mercier, A. (Eds.), Advances in Sea Cucumber Aquaculture and Management, FAO Fisheries Technical Paper No. 463. FAO of the United Nations, Rome, Italy, pp. 173-179.

Blankenship, H.L., Leber, K.M. 1995. A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15, 167-175.

Blaxter, J.H.S. 1994. Summary of symposiumon sea ranching of cod and other marine fish species. Aquacult. Fish. Manage. 25, 259-264.

Blaxter, J.H.S. 2000. The Enhancement of Marine Fish Stocks. In: Southward, A.J., Tyler, P.A., Young, C.M., Fuiman, L.A. (Eds.), Advances in Marine Biology. Academic Press, London, pp. 1-54.

Brown, C., Day, R.L. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish and Fisheries 3, 79-94.

Cabaitan, P.C. and C. Conaco. 2017. Bringing back the giants: juvenile *Tridacna gigas* from natural spawning of restocked giant clams. Coral Reefs 36:591.

Cowx, I.G. 1994. Stocking strategies. Management Ecology 1, 15-30.

Crowe, T.P., Lee, C.L., McGuinness, K.A., Amos, M.J., N'Guyen, F., Tetelepta, J., Dangeubun, J., Dwiono, S.A.P., Makatipu, P.C., Manuputty, J., N'Guyen, F., Pakoa, K., Tetelepta, J. 2002. Experimental evaluation of the use of hatchery-reared juveniles to enhance stocks of the topshell *Trochus niloticus* in Australia, Indonesia and Vanuatu. Aquaculture. 206, 175-197.

Drawbridge, M.A. 2002. The role of aquaculture in the restoration of coastal fisheries, In: Costa-Pierce, B.A. (Ed.), Ecological Aquaculture, The Evolution of the Blue Revolution. Blackwell Science, Oxford, pp. 314-336.

FAO. 2016. Illegal, unreported and unregulated fishing. FAO Document I6069E/1/09.16 (http://www.fao.org/3/a-i6069e.pdf)

FAO. 2019. Fishery and Aquaculture Statistics. Global capture production 1950-2017 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2019

Fujii, T. 2016. Potentials and Limitations of Stock Enhancement Programs in Japan. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp. 136-139.

Fushimi, H. 2001. Production of juvenile marine finfish for stock enhancement in Japan. Aquaculture 200, 33-53.

Fushimi, H. 2016. Improvement of Stocking Efficiencies. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp 18-24.

Garaway, C.J., Arthur, R.I., Chamsingh, B., Homekingkeo, P., Lorenzen, K., Saengvilaikham, B., Sidavong, K. 2006. A social science perspective on stock enhancement outcomes: Lessons learned from inland fisheries in southern Lao PDR. Fisheries Research. 80, 37-45.

Gomez E.D., Mingoa-Licuanan S.S. (2006) Achievements and lessons learned in restocking giant clams in the Philippines. Fish Res 80:46–52.

Harada, Y., Matsumiya, Y. 1992. A theoretical study on resource enhancement by stocking, with special reference to its intergenerational effects. Nippon Suisan Gakkaishi 58, 1833-1842.

Howell, B.R., Moksness, E., Svasand, T. 1999. Stock Enhancement and Sea Ranching. Fishing News Books, Oxford, United Kingdom.

Jia, J. S., and J. X. Chen. 2001. Sea farming and sea ranching in China. FAO Fish. Tech. Paper, 418.

Juinio, M.A.R., Menez, L.A.B., Villanoy, C.L., Gomez, E.D. 1989. Status of giant clam resources in the Philippines. J. Mollus. Stud. 55, 431–440.

Juinio-Meñez, M.A. S. Paña, G.M. de Peralta, T.O. Catbagan, R.D. Olavides,C.A. Edullantes, B.D. Rodriguez. 2012. Establishment and management of communal sandfish (*Holothuria scabra*) sea ranching in the Philippines. In: C.A. Hair, T.D. Pickering, D. Mills, eds. Asia-Pacific tropical sea cucumber aquaculture. Proceedings of an International symposium held in Noumea, New Caledonia, 15-17 February 2011. ACIAR Porc. No. 136. Australian Center for International Agricultural Research, Canberra. 121-127.

Juinio-Meñez, M.A., J.C. Evangelio, R. D. Olavides, M.A. Paña, G. M. de Peralta, C. M. Edullantes, B. D. Rodriguez, I.L. Casilagan. 2013. Population dynamics of cultured *Holothuria scabra* in a sea ranch: Implication for Stock Restoration. Rev. in Fish. Sci. 21(3-4): 424-432.

Juinio-Meñez. M.A. 2016. Approaches in rebuilding sea urchin and sea cucumber populations in the Philippines. In: Hajime, K., *et al.* (Eds.) Consolidating the Strategies for Fisheries Enhancement in Southeast Asia. SEAFDEC/TD. pp 161-164.

Kitada, S. 1999. Effectiveness of Japan's stock enhancement programmes: Current perspectives. In: Howell, B.R., Moksness, E., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching. Fishing News Books, Oxford, United Kingdom, pp. 103-131.

Kitada, S., and Kishino, H. 2006. Lessons learned from Japanese marine finfish stock enhancement programmes. Fisheries Research. 80, 101-112.

Kitada, S. 2018. Economic, ecological and genetic impacts of marine stock enhancement and sea ranching: A systematic review. Fish and Fisheries. 19, 511-532.

Kitada, S., Nakajima, K., Hamasaki, K., Shishidou, H., Waples, R.S., Kishino, H. 2019. Rigorous monitoring of a large-scale marine stock enhancement program demonstrates the need for comprehensive management of fisheries and nursery habitat. Scientific Reports. 9, 5290

Le Vay, L., Lebata, M.J.H., Walton, M., Primavera, J., Quinitio, E., Lavilla-Pitogo, C., Parado-Estepa, F., Rodriguez, E., Ut, V.N., Nghia, T.T., Sorgeloos, P., Wille, M. 2008. Approaches to stock enhancement in mangrove-associated crab fisheries. Reviews in Fisheries Science 16, 72-80.

Lebata-Ramos M.J. and Doyola-Solis E. 2016. Fishery Resource Enhancement: An Overview of the Current Situation and Issues in the Southeast Asian Region. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp. 124-128.

Leber, K.M. 1999. Rationale for an experimental approach to stock enhancement. In: Howell, B.R., Moksness, E., Svåsand, T. (Eds.), Stock Enhancement and Sea Ranching. Fishing News Books, Oxford, pp. 63–73.

Leber, K.M. 2004. Marine stock enhancement in the USA: status, trends and needs. In: Leber, K.M., Kitada, S., Blankenship, H.L., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Publishing Ltd., Oxford, United Kingdon, pp. 11-24.

Lenanton, R.C., Ayvazian, S.G., Dibden, C., Jenkins, G., Sarre, G. 1999. The use of stock enhancement to improve the catch rates of black bream, *Acanthopagrus butcheri* (Munro) for Western Australian recreational fishers. In: Howell, B.R., Moksness, E., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching. Fishing News Books, Oxford, United Kingdom, pp. 219–230.

Liao, I.C. 1999. How can stock enhancement and sea ranching help sustain and increase coastal fisheries? In: Howell, B.R., Moksness, E., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching. Fishing News Books, Oxford, pp. 132-149.

Liao, I.C., Su, M.S., Leano, E.M. 2003. Status of research in stock enhancement and sea ranching. Reviews in Fish Biology and Fisheries 13, 151-163.

Lorenzen, K., Agnalt, A.-L., Blankenship, H.L., Hines, A.H., Leber, K.M., Loneragan, N.R., Taylor, M.D. 2013. Evolving context and maturing science: Aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. Reviews in Fisheries Science. 21, 213-221.

Lorenzen, K., Garaway, C.J., Chamsingh, B., Warren, T.J. 1998. Effects of access restrictions and stocking on small water body fisheries in Laos. Journal of Fish Biology. 53, 345-357.

Lorenzen, K., Leber, K.M., Blankenship, H.L. 2010. Responsible approach to marine stock enhancement: An update. Reviews in Fisheries Science. 18, 189-210.

Manajit, N., Theparoonrat, Y., Amornpiyakrit, T., Yingyuad, W. 2016. Habitat conservation and resources enhancement in seagrass beds in Sriboya Island, Krabi Province, Thailand. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp 117-120.

Masuda, R., Tsukamoto, K. 1998. Stock enhancement in Japan: Review and perspective. Bulletin of Marine Science. 62, 337-358.

Mingoa-Licuanan, S.S. and E. D. Gomez. 2002. Giant clam conservation in Southeast Asia. Tropical Coasts. 9(2): 24-56.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000. Effect of aquaculture on world fish supplies. Nature. 405, 1017-1024.

Okamoto, K. 2004. Juvenile release and market size recapture of the swimming crab *Portunus trituberculatus* (Miers) marked with coded wire tags. In: Leber, K.M., Kitada, S., Blankenship, H.L., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Publishing Ltd., Oxford, United Kingdom, pp. 181-186.

Okuzawa, K., Maliao, R., Quinitio, E., Buen-Ursua, S., Lebata, J., Gallardo, W., Garcia, L., Primavera, J. 2008. Stock enhancement of threatened species in Southeast Asia. Reviews in Fisheries Science. 16, 394-402.

Oshima, Y. 1993. History of technical development in stock enhancement and aquaculture. Midori Shobo, Tokyo, Japan.

Primavera, J.H. 2005. Mangroves, fishponds, and the quest for sustainability. Science. 310, 57-59.

Primavera, J.H., Altamirano, J.P., Lebata, M.J.H.L., delos Reyes, A.A., Pitogo, C.L. 2007. Mangroves and shrimp pond culture effluents in Aklan, Panay Is., Central Philippines. Bulletin of Marine Science. 80, 795-804.

Purcell, S.W., Hair, C.A., Mills, D.J. 2012. Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. Aquaculture. 368–369, 68-81.

Radtke, H.A., Davis, S.W. 2000. Economic feasibility of salmon enhancement propagation programs, in: Knudson, E.E., Steward, C.R., MacDonald, D.D., Williams, J.E., Reiser, D.W. (Eds.), Sustainable Fisheries Management: Pacific Salmon. CRC Press, Boca Raton, pp. 381-392.

Ravago-Gotanco, R., Kim, K.M. 2019. Regional genetic structure of sandfish *Holothuria* (Metriatyla) *scabra* populations across the Philippine archipelago. Fish Res. 209, 143-155.

Ray, M., Stoner, A.W., O'Connell, S.M. 1994. Size-specific predation of juvenile queen conch, *Strombus gigas*: iImplications for stock enhancement. Aquaculture 128, 79-88.

Rowland, S.J. 1994. Stocking of freshwater fishes and policy in New South Wales,. In: Prokop, F. (Ed.), Translocation Issues in Western Australia. Fisheries Department of Western Australia, Fisheries Management Paper 83, pp. 50-62.

Salayo, N.D., Azuma, T., Castel, R.J.G., Barrido, R.T., Tormon-West, D.H.M., Shibuno, T. 2020. Stock enhancement of abalone, *Haliotis asinina*, in multi-use buffer zone of Sagay Marine Reserve in the Philippines. Aquaculture. 523, 735138.

Salvanes, A.G.V. 2001. Ocean ranching. In: Steele, J., Turekiane, K.K., Thorpe, S.A. (Eds.), Encyclopedia of Ocean Sciences, Academic Press, pp. 1973-1982

Siriraksophon, S. 2016. Fisheries refugia: A regional initiative to improve the integration of fisheries and habitat management. In: Kawamura H., Iwata T., Theparoonrat Y., Manajit N., and Sulit V.T. (Eds). Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia. SEAFDEC/TD. pp 80-92.

Stickney, R.R. 1996. Aquaculture in the United States: A historical survey. John Wiley and Sons, New York, USA.

Svåsand, T., Kristiansen, T. S., Pedersen, T., Salvanes, A. V., Engelsen, R., Naevdal, G., Nødtvedt, M. 2000. The enhancement of cod stocks. Fish and Fisheries, 1, 173–205.

United Nations. 2019. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423). United Nations, New York.

United Nations. 2015. Transforming Our World: The 2030 Agenda for Sustainable Development (A/RES/70/1). United Nations, New York.

Wang, Y.G., Haywood, M.D.E. 1999. Size-dependent natural mortality of juvenile banana prawns *Penaeus merguiensis* in the Gulf of Carpentaria, Australia. Marine and Freshwater Research 50, 313-317.

Wang, Q.Y., Zhuang, Z.M., Deng, J.Y., Ye, Y.M. 2006. Stock enhancement and translocation of the shrimp *Penaeus chinensis* in China. Fisheries Research 80, 67-79.

Welcomme, R.L., 1992. A history of international introductions of inland aquatic species. FAO Fisheries Technical Paper No. 294. FAO, Rome, p. 318 pp.

Welcomme, R.L., Bartley, D.M. 1998. Current approaches to the enhancement of fisheries. Fisheries Management and Ecology 5, 351-382.

Yokoyama, H. 2013. Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages - Potential for integrated multi-trophic aquaculture. Aquaculture. 372-375, 28-38.

Yulianto, I., Hammer, C., Wiryawan, B., Palm, H.W. 2019. Cost-benefits of grouper *Epinephelus fuscoguttatus* (Forsskål, 1775) stock enhancement and sea-ranching in Indonesia. Asian Fisheries Science. 32, 124-130.

Ziemann, D.A. 2001. The potential for the restoration of marine ornamental fish populations through hatchery releases. Aquar. Sci. Conserv 3, 107-117.

Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V., Zohar, Y. 2005. Large-scale production of the blue crab *Callinectes sapidus*. Aquaculture 244, 129-139

Potential Use of Agricultural Wastes in Aquafeed Production

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Abstract

Disposal of agricultural wastes are posing environmental hazards which leads to efforts of efficiently utilizing them. This study surveyed a sugar central and a fruit processing plant to collect data on the volume of wastes from representative agricultural crops (e.g. mango, citrus, pineapple, sugarcane, papaya and soybean) in the Philippines during the 2012–2013 and 2014–2015 seasons, respectively. Their potential use in aquafeed was examined in terms of nutritional quality, presence of anti-nutritional factors (ANFs) and pesticide residues. About 40 to 60 % of agricultural wastes generated after processing were peels, pulps or brans, seeds, bagasse, molasses and okara. Most of the agricultural wastes had high levels of fiber and carbohydrate, and low levels of protein, although okara (25 % crude protein) and citrus by-products (11–16 % crude protein) showed acceptable nutritional quality. ANFs such as lignin are largely present in all agricultural wastes, whereas high levels of phenols, and saponins and alkaloids were found in mango seeds and mango peels, respectively. Pesticide residues were detected only in mango and citrus peels but at levels below the maximum residue limits of FAO Codex Alimentarius. From both nutritional and environmental perspectives, agricultural wastes have potential use in aquafeed production but their suitability should be further elucidated in diets for omnivorous fish species such as tilapia.

Keywords: agricultural wastes; anti-nutritional factors; pesticide residues

Introduction

The increasing growth of the aquaculture sector has undeniably intensified the demand for compound feeds. The projected feed usage of major fed species in 2020 amounted to 65.4 million metric tons (Tacon and Metian, 2015). Commercial feeds are expensive for the smallholder farmers, mainly attributed to fishmeal (FM) and fish oil (FO), which are included at higher proportions. Therefore, focusing on readily available alternative feed sources which could provide affordable options in feed formulation is necessary.

The Philippines has 13 million hectares dedicated to farming of agricultural crops such as mango, coconut, banana, pineapple, citrus and sugarcane. Tremendous amounts of agricultural wastes are produced after the processing of these crops that could be utilized for animal feed production. The most common wastes include peels, seeds, pulps or bran, bagasse, and molasses. However, limited information is available on the availability and utilization of these agricultural wastes in the aquaculture industry. Disposal of these agricultural

wastes may cause adverse environmental impacts. Efforts to efficiently utilize them as raw material for aquafeed production would be beneficial for the development of the feed industry (Aya, 2017).

Some agricultural wastes have been successfully utilized in diets for Pacific white shrimp *Litopenaeus vannamei* (Forster *et al*., 2010), Nile tilapia *Oreochromis niloticus* (El-Sayed, 1991; Akegbejo-Samsons *et al*. 2006; El-Sayed *et al*., 2010; El-Saidy, 2011) and rohu *Labeo rohita* (Abani Deka *et al*., 2003). The suitability of agricultural wastes in fish feeding would depend on their nutritional composition, presence of anti-nutritional factors (ANFs), and food safety issues such as pesticide residues. While these factors may limit higher inclusion of agricultural wastes in aquafeed, appropriate processing techniques may be employed to improve their nutritional value and to reduce their ANFs contents.

To ascertain the potential use of representative agricultural wastes in aquafeeds, this study surveyed a sugar central and a fruit processing plant to collect data on the volume of agricultural wastes produced during the 2012–2013 and 2014–2015 seasons, respectively. Interviews were also conducted at relevant government agencies to gather information on agricultural waste management and utilization projects in the aquaculture sector. The proximate composition, presence of ANFs and pesticide residue levels were also examined to determine the suitability of agricultural wastes in aquafeed production for environmental and economic reasons.

Materials and methods

A review of secondary data, interviews and visits to the government offices (e.g. Philippine Statistics Authority, Bureau of Plant Industry, Sugarcane Regulatory Administration and the Bureau of Agricultural Research), sugar central (Balayan, Batangas, Philippines) and fruit processing plant (Guiguinto, Bulacan, Philippines) were conducted to obtain relevant data and information on the 1) production statistics of major agricultural crops; and 2) volume of waste production, utilization and treatment. The volume of agricultural wastes produced from sugarcane industry covered the 2012-2013 season, whereas those from the fruit processing plant were obtained during the 2014-2015 production season.

Agricultural wastes (e.g. sugarcane baggase, mango peel and kernel, citrus peel, pulp and seeds) were collected directly from their respective processing plants. Okara (a soy byproduct), banana and pineapple peels were obtained from cottage industries and wet market, respectively in Binangonan, Rizal, Philippines and transported to the SEAFDEC/AQD laboratory, also in Binangonan, for further processing. Agricultural wastes were rinsed or washed (when appropriate), oven-dried at 60°C, ground and stored for further analysis. The proximate composition (crude protein, crude fat, crude fiber, crude ash and moisture) was determined using standard methods (AOAC, 2000). They were also examined for the presence of anti-nutritional factors (ANFs; e.g. tannin, phenol, saponin, lignin and alkaloid) and pesticide residues (e.g. organochlorines, pyrethroids and organophosphates). Samples for the analyses of ANFs and pesticide residues were submitted to the Philippines' Adamson University Technology Research and Development Center and the Department of Agriculture - Bureau of Plant Industry National Pesticide Analytical Laboratory, respectively.

Results

Agricultural wastes and their utilization

Table 1 shows the volume of production among major agricultural crops in the Philippines. Sugarcane registered the highest volume of production, followed by coconut, banana, pineapple, mango and citrus. The estimated quantity of wastes generated from the major crop industries (e.g. mango, pineapple, citrus and sugarcane) after processing are presented in **Table 2**, including that of okara.

Table 1. Volume of production of agricultural crops in the Philippines, 2013[1]

Crops	Production (in MT)[1]
Sugarcane	24,584,820
Coconut	15,344,000
Banana	8,645,749
Pineapple	2,458,422
Mango	816,199
Citrus	164,060
Soybean[2]	1.5-2.5

[1]Philippine Statistics Authority 2014

[2]Data accessed from http://businessdiary.com.ph/4551/soybean-production-guide/; production from NW and Central Luzon and Surigao del Sur; unit expressed as t/ha

Table 2. Volume of production and wastes among major agricultural crops in the Philippines, 2013

Crops	Volume use in production (in tons)	Volume of wastes produced (in tons)	Type of Wastes
Mango[1]	3,600-4,800	1,800-2,400	peel, kernel, seeds
Calamansi[1]	2,700-3,150	1,755-2,047	peel, seeds, pulp
Pineapple[1]	210	105-126	bran, peels
Sugarcane[2,3]	24,859,027	9,175,010	bagasse, filter cake,
	470,229	172,544	Molasses
Papaya[1]	60	30-36	pomace, seeds
Soybean[4]	1.5	1.5	okara

[1]Data obtained from a Fruit Processing Plant in Guiguinto, Bulacan

[2]SRA Annual Synopsis: Philippine Sugar Factories' Production and Performance Data 2012-2013

[3]Data obtained from a Sugar Central in Balayan, Batangas

[4]Data obtained from cottage industries in Binangonan, Rizal

Nutrient composition of agricultural wastes

The proximate composition of agricultural wastes is shown in **Table 3**. The moisture content ranged from 1.38 % to 8.09 %. The highest protein content was found in okara (25.31 %) followed by citrus by-products (9.28 %–15.71 %) and the lowest protein content was found in sugarcane bagasse (2.83 %) and mango kernel (2.53 %). Citrus seeds had the highest fat content followed by okara and banana peel. Except for mango kernel, sugarcane bagasse, okara and fruit wastes had the highest fiber levels. The highest NFE was determined in mango (78.06 % to 88.34 %) and pineapple (81.41 %) wastes and the highest ash content in banana peel (14.81). The highest gross energy content was estimated in citrus seeds (23 kJ g^{-1}) followed by okara (18.77 kJ g^{-1}) and other fruit wastes (14.11 to 16.68 kJ g^{-1}). The lowest gross energy content was determined in sugarcane bagasse (10.20 kJ g^{-1}). The potential digestible energy for fish also showed the same trend as gross energy.

All agricultural wastes contained ANFs (e.g. tannin, phenols, saponin, lignin and alkaloid) (**Table 4**). Citrus seeds and mango kernel had the highest tannin levels (0.28 and 0.29 %) whereas okara and pineapple peel had the lowest at 0.11 %. Okara and citrus seeds were detected to contain saponin levels at 0.25 and 0.09 %, respectively. All contained high levels of lignin particularly citrus seeds (47.45 %). Mango kernel was characterized by a high level of phenol (11.31 %) and alkaloid (3.90 %).

Pesticide residues

Among the agricultural wastes analyzed, mango peel and citrus peel were found to contain pesticide residues (**Table 5**). Lamba-cyhalothrin, an insecticide which belongs to a group of chemicals called pyrethroids, was found in mango peels at 0.14 mg/kg. Another insecticide, chlorpyrifos was detected in citrus peels at 0.04 mg/kg.

Discussion

Agricultural wastes are the materials left after the production and processing of agricultural products (Obi *et al*., 2016). In the Philippines, many of these agricultural wastes were not utilized and their treatment and disposal can be costly. The huge amount (about 40–60 %) of wastes from agricultural crops, as surveyed in this study albeit from a very limited sources, suggest that available source of alternative feed sources is not a problem. Most of these agricultural wastes have varied industry uses such as in the development of functional feed ingredient or high-value products using biotechnological approaches, organic fertilizers, or as supplemental feeds or feedstuffs in the animal (e.g. livestock and poultry) feeds. Converting these wastes into potential feed ingredients for fish culture can also provide additional benefits to its utilization. However, several factors such as the nutritional aspects, including the presence of anti-nutritional factors (ANFs) and levels of pesticide residues shall be considered to evaluate their suitability in aquafeed production.

The agricultural wastes analyzed in this study generally contain low levels of protein and high levels of carbohydrate (as nitrogen free extract) and fiber. Only okara have the highest acceptable crude protein content (25 %) suggesting its potential use as a protein source, followed by citrus by-products which contain 9 to 11 % crude protein. High lipid contents found in citrus seeds, okara and banana peels suggest that these wastes could be an alternative sources of quality plant lipids. Extraction

Table 3 . Proximate composition (% dry matter) and energy content for fish (kJ g^{-1}) of some representative agricultural wastes in the Philippines, 2013

Agricultural wastes	Moisture	Crude Protein	Crude Fat	Crude Fiber	NFE[1]	Ash	Gross energy[2]	Potential digestible energy for fish[3]
Sugarcane bagasse	6.27	2.83	0.46	33.90	55.39	7.43	10.20	6.39
Pineapple peel	6.28	4.27	0.61	9.53	81.41	4.19	15.00	9.38
Banana peel	3.19	6.44	7.41	8.02	63.33	14.81	15.17	10.10
Citrus pulp	8.09	9.28	1.30	16.00	67.32	6.11	14.09	8.86
Citrus peel	7.59	11.50	0.72	13.90	65.69	8.20	14.11	8.82
Citrus seed	1.38	15.71	31.49	8.33	39.83	4.64	23.00	17.15
Citrus, whole	2.90	11.22	2.91	10.67	69.70	5.51	15.59	9.94
Mango peel	6.25	5.93	2.15	10.95	78.06	2.93	15.44	9.79
Mango kernel	5.19	2.53	2.94	3.65	88.34	2.55	16.68	10.64
Okara meal	4.54	25.31	12.85	12.99	45.14	3.72	18.77	12.79

[1]NFE, Nitrogen Free-Extract

[2]Gross energy (kJ g^{-1}) was calculated using standard physiological values of 23.87, 39.78, and 16.87 for protein, lipid and carbohydrate, respectively (Ulloa, 2002)

[3]Potential digestible energy for fish (kJ g^{-1}) was calculated according to digestible energy coefficients of 14.6, 33.9, and 10.5 for protein, lipid and carbohydrate, respectively for channel catfish (NRC, 1977)

Table 4. Anti-nutritional factors (ANFs) present in some representative agricultural wastes in the Philippines, 2013

Agricultural wastes	ANFs (%)				
	Tannin	Phenols	Saponin	Lignin	Alkaloid
Sugarcane baggase	0.20	1.93	-	41.49	0.71
Pineapple peel	0.11	0.53	-	21.39	0.16
Banana peel	0.20	0.39	-	41.11	0.36
Citrus seeds	0.29	0.66	0.09	47.45	1.10
Citrus peel	0.20	0.71	-	31.97	0.61
Citrus pulp	0.18	0.71	-	24.94	0.15
Mango peel	0.61	ND*	3.00	29.00	16.00
Mango kernel	0.28	11.31	-	15.42	3.90
Okara	0.11	0.60	0.25	41.49	0.11

*ND, not detected

Table 5. Pesticide residue levels in some representative agricultural wastes in the Philippines, 2013

Agricultural wastes	Pesticide residue (mg/kg)*		
	Organochlorines	Pyrethroids	Organophosphates
Sugarcane baggase	< LOQ	< LOQ	< LOQ
Pineapple peels	< LOQ	< LOQ	< LOQ
Mango peels	< LOQ	Lamba-cyhalothrin = 0.14	< LOQ
Banana peels	< LOQ	< LOQ	< LOQ
Citrus peels	Chlorpyrifos = 0.04	< LOQ	< LOQ

*Limit of Quantification (LOQ) for organophosphates, organochlorines and pyrethroids is 0.01 mg/kg

of lipids from these wastes using organic solvents could further increase their nutritional quality. All except for mango seed or kernel have higher levels of crude fiber (8-34 %) exceeding the requirement (less than 7 %) in aquafeeds, thereby limiting the utilization of this ingredient. Fiber contents in aquafeeds should be maintained at less than 7 % to reduce the amount of undigested material going into the receiving waters (Gatlin, 2010). High ash content found in banana peels (more than 12 %) could result to poor digestibility and growth of fish when promoted in aquafeeds (De Silva and Anderson, 1995).

The presence of anti-nutritional factors (ANFs) in alternative feed sources is another factor that could limit their utilization in aquafeeds (Francis *et al*., 2001). All the agricultural wastes in the present study showed the presence tannins, phenols, saponins, lignin and alkaloids. Tannin contents (0.11-0.29 %) did not exceed the 0.63% level known to cause an impact on the digestibility of dry matter, protein and lipid (Pinto *et al*., 2000). In another study, tannins at 2 % inclusion rate in the diet could interfere with the digestive processes (Becker and Makkar, 1999). All the agricultural wastes

had levels of tannins below this inclusion rate. Agricultural wastes had high levels of lignin (15.42–47.45 %) which may affect protein digestibility and palatability. High levels of phenol in mango seed (11.31 %) may limit its use in fish feeding given that long-term exposure of phenols above this inclusion rate (0.002 %) have negative effect on the immune response of *Tilapia nilotica* fingerlings (Zaki and Fawzi, 2016). Saponin, which gives a bitter taste that can reduce palatability in fish diets, was found in low levels in citrus seeds (0.09 %), and are unlikely to affect fish growth due to their lower contents (Francis *et al*., 2001). High levels of saponin found in mango peels (3 %) and intermediate in okara (0.25 %), and their inclusion at higher levels in fish diets may result in low feed palatability and utilization (Tacon, 1993). Saponins above 0.15 % inclusion rate was detrimental to the growth and intestinal morphology in fish (Francis *et al*., 2001), and both okara and mango peels exceeded this level. Despite this high saponin contents, mango peels could still be considered a potential feed ingredient as they contain a concentrated amount of carotenoid pigments that are contributors to fish reproduction. Therefore, to reduce the ANF contents and maximize the inclusion in fish diets, these agricultural wastes should be processed further using biological or chemical techniques (e.g. ensiling or solid state fermentation).

Apart from their nutritional quality, pesticide residues in agricultural wastes should be examined before finally considering them in fish feeds. Pesticide residues such as lamba-cyhalothrin and chlorpyrifos were detected in mango and citrus peels, respectively, and residue levels were found to exceed the limit of quantification of the Philippines' Department of Agriculture - Bureau of Plant Industry National Pesticide Analytical Laboratory. Lamba-cyhalothrin is an insecticide which belongs to a group of chemicals called pyrethroids, and chlorpyrifos is also an insecticide. However, the detected pesticide residue levels were still lower than the maximum residue limits (MRLs) adopted by the FAO Codex Alimentarius for mango (0.2 mg/kg) and citrus (1 mg/kg) in 2009 and 2013, respectively (http://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/). This show that the use of mango and citrus peels may not pose any adverse impact on fish health condition when promoted in aquafeed production although further studies are needed.

Okara have already been used as a protein source replacing fishmeal at levels up to 750 g kg^{-1} in mono-sex Nile tilapia fingerlings diets (El-Saidy, 2011). In contrast, our results showed that okara have been included in mixed-sex Nile tilapia fingerlings at levels up to 300 g kg^{-1} diet (Aya *et al*., unpublished data). The low inclusion levels of okara in our study may be due to high fiber content and the presence of ANFs in this ingredient.

Conclusion

Agricultural wastes have very low or no commercial value, and are readily available in large quantities, making them potential feed ingredients in aquafeeds. The suitability of these agricultural wastes was evaluated based on nutritional composition, presence of anti-nutritional factors (ANFs) and pesticide residues. Because most agricultural wastes contain ANFs and high fiber content, appropriate processing treatments to increase their nutritional value should be applied and their suitability tested in diets for omnivorous fish species such as tilapia to promote low-cost feeds for fish culture.

Acknowledgment

The study was supported by the Government of Japan – Trust Fund 6 under the study code: 8300-B-RD-FD0415. The authors thank the staff of the sugar central, fruit processing plant and government offices for sharing their data with the authors and Mr. Nemencio Olorvida for his assistance during field collection.

References

Abani D, Sahu NP, Jian KK. 2003. Utilization of fruit processing wastes in the diet of *Labeo rohita* fingerling. Asian-Australasian Journal of Animal Sciences 16: 1661-1665

Akegbejo-Samsons Y, Omoniyi T. 2006. Evaluation of pineapple crush waste meal as an energy feedstuff in the diets of tilapia, *Oreochromis niloticus*. Nigerian J Anim Prod 33: 308-312

AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis, 17th edition. AOAC, Washington, District of Columbia, USA.

Aya FA. 2017. Utilizing alternative ingredients in aquafeeds for sustainable aquaculture. In: Fish for the People. Vol. 15 No. 3. Southeast Asian Fisheries Development Center, Bangkok, Thailand; pp 37-44

Becker K, Makkar HPS . 1999. Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpio* L.). Aquaculture 175: 327-335

El-Saidy DMSD. 2011. Effect of using okara meal, a by-product from soymilk production as a dietary protein source for Nile tilapia (*Oreochromis niloticus* L.) mono-sex males. Aquaculture Nutrition 17: 380-386

El-Sayed AFM. 1991. Evaluation of sugarcane bagasse as a feed ingredient for the tilapias *Oreochromis niloticus* and *Tilapia zilii*. Asian Fisheries Science 4: 53-60

El-Sayed SA, El-Kholy M, Eleraky WA, Soliman MH. 2010. Effect of partial replacement of yellow corn with dried citrus pulp in Nile tilapia diets on growth performance, nutrient digestibility and immune status. Engormix.com

De Silva SS, Anderson TA. 1995. Fish Nutrition in Aquaculture. 1st edn. Chapman and Hall, 2-6, Boundry Row, London, SE 1 8HN, UK

Food and Agriculture Organization of the United Nations and World Health Organization. FAO/WHO Food Safety Standards. Codex Alimentarius. http://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/

Foster IP, Dominy WG, Conquest LD, Ju ZY, Grey M. 2010. Use of agriculture byproducts in diets for Pacific white shrimp *Litopenaeus vannamei*. En: Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0. Universidad Autónoma de Nuevo León, Monterrey, México, pp 366-392

Francis G, Makkar H, Becker K. 2001. Antinutritional factors present in plant-derived alternate fish ingredients and their effects in fish. Aquaculture 199: 197-227

Gatlin DM. 2010. Principles of Fish Nutrition. Southern Regional Aquaculture Center. SRAC Publication No. 5003.

National Research Council. 1977. Nutrient requirements of warmwater fishes. National Academy of Science, Washington D.C., USA, 77 p

Obi FO, Ugwuishiwu BO, Nwakaire. 2016. Agricultural waste concept, generation, utilization and management. Nigerian Journal of Technology 35: 957-964

Philippine Statistics Authority. 2014. Selected statics on Agriculture 2014. https://psa.gov.ph/

Pinot LGQ, Pezzato LE, Miranda EC, Barros MM, Furuya WM. 2000. Ação do tanino na digestibilidade de dietas pela tilápia-do-nilo (*Oreochromis niloticus*). Acta Scientiarum 22: 677-681

Soybean Production Guide (https://businessdiary.com.ph/4551/soybean-production-guide/). Accessed: 09 January 2020

Sugar Regulatory Administration. 2014. Annual Synopsis of Philippine Raw Sugar Factories Production and Performance Data 2012-2013

Tacon. 1993. Feed ingredients for warmwater fish: Fishmeal and other processed feedstuffs. FAO Fisheries Circular No. 856. Rome, Italy. 64 p.

Tacon A, Metian M. 2015. Feed matters: Satisfying the feed demand of aquaculture. Reviews in Fisheries Science and Aquaculture 23: 1-10.

Ulloa Rojas JB. 2002. Use of coffee pulp as feed ingredient for tilapia culture. PhD thesis, Wageningen University, Wageningen, The Netherlands, 133 p.

Zaki MS, Fawzi OM. 2016. Phenol toxicity affected *Tilapia nilotica* fish. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7: 766-770

Ammonia, Phosphate, Total Suspended Solid and Chlorophyll *a* Removal in Mangrove Habitat Receiving Shrimp Pond Effluents

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Abstract

Diseases continue to devastate the shrimp industry. One culture system that has the potential to abate disease occurrence, improve shrimp survival and environment-friendly is aquasilviculture. Aquasilviculture is the culture of aquatic organism with mangroves inside the pond (mixed system) or in the receiving environment (separate). A previous study reported that the presence of mangroves in the receiving environment enhances shrimp survival via an improved incoming water quality. The present study determined the time required for a mangrove habitat to remove nutrients from shrimp (*Penaeus monodon*) farm effluents and the factors affecting mangrove efficiency to remove nutrients. Results showed that ammonia, phosphate, chlorophyll a and total suspended solids (TSS) were fluctuating but statistically lower in water drained into mangrove habitat (Mangrove to Pond Area Ratio, (MPR)=2:1 and MPR=4:1) compared to area without mangroves (MPR=0). At MPR=4:1, ammonia is removed from the water after 3 days; TSS after 2 days; phosphate and chlorophyll a after 7 days. At MPR=2:1, only ammonia can be efficiently removed after 3 days. These results further showed that the type of nutrient and MPR affect the efficiency of mangroves to remove nutrients from shrimp farm effluents.

The growth of plants in areas receiving and not receiving shrimp farm effluents were compared by measuring the monthly increase in the seedling height and the increase in the stem length between two nodes in saplings and trees. After 3 months, increase in growth was greater in plants in area receiving shrimp farm effluents compared to those not receiving, except for the seedlings. This indicates that mangroves purify the water by nutrient uptake as supported by the data showing greater increase in stem length in saplings and trees.

Introduction

Diseases continue to devastate the shrimp industry. One culture system that has the potential to abate disease occurrence and improve shrimp survival is aquasilviculture. Aquasilviculture is the culture of aquatic organism with mangroves. There are 2 types of aquasilviculture system: mixed, wherein mangroves are inside the culture pond; and separate, wherein the mangroves are in the receiving environment (Primavera

et al., 2000). Less disease incidence is reported in areas with mangroves (Belak *et al*., 1999). No Whitespot Syndrome Virus (WSSV) outbreak was observed in shrimp cultured in pens inside mangrove habitat despite WSSV outbreak in adjacent ponds (Lebata *et al*., 2012). High mangrove-to-pond area ratio (MPR) is a WSSV protective factors (Tendencia *et al*., 2011). One-way mangrove influences disease occurrence could be via an improved water quality (Tendencia *et al*., 2012). Higher phosphorus (P) and nitrogen (N) removal percentage in ponds with mangrove seedlings than in ponds without seedlings were observed in the Pacific whiteleg shrimp *Litopenaeus vannamei* experimental ponds (Moroyoqui-Rojo *et al*., 2012). Several studies have reported the capability of mangroves to remove nutrients from pond effluents and this depends on the ratio of mangrove forest: shrimp pond area (MPR) (Robertson and Philipps, 1995; Shimoda *et al*., 2005, 2007; Primavera *et al*., 2007). Reported MPR varied depending on the type of nutrient to be removed and the type of system. An MPR of 4:1 was reported to establish a healthy ecosystem (Saenger *et al*., 1983). In a separate system, an MPR of 2 to 22:1 is required to filter the N and P loads (Robertson and Philipps, 1995); while an MPR of 0.04 to 0.12: 1 is required to completely remove the dissolved inorganic nitrogen (Rivera-Monroy *et al*.,1999). In mixed mangrove and pond systems, MPR of 2.1 to 5.2: 1 is required to remove the N remaining in the aquaculture pond (Shimoda *et al*., 2007), while 6.2 to 8.9:1 is required to fully process the P (Shimoda *et al*., 2005). Impounded mangroves of 1.8-5.4 ha are required to remove the nitrate wastes from 1 ha of shrimp pond (Primavera *et al*., 2007).

Other importance of mangroves, aside from being a biofilter is that some parts of the mangrove tree, particularly the leaves, have antimicrobial properties (Sudheer *et al*., 2011). The leaves may also provide a suitable substrate for the growth of favourable periphytic biofilm (Tran & Yakupitiyage, 2005; Gatune *et al*., 2012). Furthermore, leaf litter enriches the environment during decomposition. The presence of mangrove can also mitigate the effects of climate change through carbon sequestration (Donato *et al*., 2011) and lowers air and water temperature (Le, 2006).

This paper determined the time required for a mangrove habitat to remove nutrients (i.e. N, P) from shrimp farm effluents; and, the factors affecting mangrove efficiency to remove nutrients.

Materials and method

Two experiments were done using earthen ponds. The number, size and purpose of the ponds used in the two experiments are presented in **Table 1.** Aeration was provided using a paddle wheel. No water change was implemented in the entire duration of the experiments. Water loss due to evaporation was replenished as needed using water from a reservoir to maintain 1.0 m water depth. Shrimp were fed commercial pellet (please see **Table 2** for the proximate analysis) ad libitum until termination.

In the first experiment, MPR=2:1 vs MPR=0, *P. monodon* postlarvae (ABW= 0.006) were stocked at 20 ind/m^2 in a 700 m^2 earthen pond. Pond effluents were drained into 2 types of receiving environment: with mangroves (area=1400 m^2; MPR=2:1) and without mangroves (4 m^2; MPR=0) after 50 days of shrimp culture. MPR used was lower than the reported MPR needed for a healthy ecosystem, thus, this experiment was not replicated.

In the second experiment, MPR=4:1 vs MPR=0, *P. monodon* postlarvae

Table 1. Number, size and purpose of the ponds used in the two experiments

Pond No	# of units	Description	Purpose	Size	
				Experiment 1	Experiment 2
1	1	With mangrove	Settling pond*	1.4 ha	1.4 ha
2	1	Without mangrove	Culture pond	0.7 ha	0.35ha
3	1	With few mangrove	Reservoir	0.6 ha	0.6 ha
4	1	Without mangrove	Settling Pond*	0.1 ha	0.1 ha

*Received pond effluents

Table 2. Proximate analysis of the commercial pellet used to feed the experimental shrimp

Composition	Amount (dry weight basis)
Crude protein (% min)	47
Crude fat (% min)	8
Crude fiber (% max)	3
Crude ash (% max)	16
Moisture (% max)	12

(ABW=0.005) were stocked at 25 ind/m^2 in a 350 m^2 earthen pond. Pond effluents were drained into 2 types of receiving environment: with mangroves (area=1400 m2; MPR=4) and without mangroves (4 m2; MPR=0) after 120 days of shrimp culture. The experiment was replicated three times using the same set of ponds.

In both experiments, water samples for ammonia and phosphate analyses were taken from the receiving environments daily on the first week after water drain and weekly thereafter until the 4th week. Water samples for chlorophyll a and total suspended solids (TSS) analyses were taken from the receiving environment on days 0, 1, 6, 7, 14, 21, 28 and days 0, 1, 2, 3, 5, 7, 14, 21, 28, respectively. Water parameters were measured following procedures described in the 23rd edition of the Standard Methods for the Examination of Water and Wastewater (2017). Ammonia-nitrogen content was measured using the phenate method (APHA AWWA WEF 4500-NH_3); phosphate, using the ascorbic acid method (APHA AWWA WEF 4500-P); TSS, filtration-oven drying method (APHA AWWA WEF 2540); and chlorophyll a using spectrophotometry (APHA AWWA WEF 10200).

Mangrove community structure (MCS) was done before the start of the first trial of Experiment 2 and before the start of every trial of the experiment and after the third trial. MCS was done approximately 1 month after draining shrimp farm effluent. Density was calculated using a formula of Krebs (1999):

Density = Number of individuals per plant type / the area of the sample (ha)

In a separate investigation, the effect of shrimp farm effluent on mangrove plant was done by measuring plant growth in areas receiving and not receiving shrimp farm effluents. Plant growth in the two environments were compared by measuring the monthly increase in seedling height and stem length between two nodes in saplings and trees. The measurement was done monthly for 3 months on 10 trees, 10 saplings, and 10 seedlings, each site, that were tagged and labelled, so that the same plant is measured during samplings.

Statistical analysis

Water nutrient levels were analysed using ANOVA and repeated measures using SPSS V 23.

Results

In Experiment 1, ammonia, phosphate, chlorophyll a and total suspended solids were fluctuating but generally lower, especially the ammonia level, in water drained into habitat with mangroves compared to those without mangrove (**Table 3**). Ammonia was not detected in habitat with mangrove 3 days after draining; phosphate after 5 days; while levels of these nutrients in ponds without mangrove remained high until after 14 days. Using repeated measures, ammonia and chlorophyll a were significantly reduced in the receiving environment with mangroves (MPR=2:1) (**Table 4**).

In Experiment 2, ammonia, phosphate, chlorophyll a and total suspended solids (TSS) were fluctuating (**Table 5**). Phosphate was not detected in habitat with mangrove 5 days after draining; TSS after 28 days. Chlorophyll a was reduced three times after 28 days. TSS in the receiving environment without mangrove increased after 2, 5, and 28 days of incubation. Phosphate was removed from both environments. Using repeated measures, significantly lower levels of ammonia, total suspended solid and chlorophyll a were observed in the habitat with mangroves that received shrimp farm effluents compared to that without mangrove (**Table 6**).

Results of the mangrove community structure is presented in **Table 7**. The number of trees, saplings and seedlings increased after receiving shrimp pond effluents after each experimental trial. In the investigation of the effect of pond effluent on mangrove, after 3 months, increase in plant growth was greater in area receiving shrimp farm effluents compared to those not receiving, except for the seedlings (**Table 8**).

Discussion

This study confirms previous reports that mangroves are capable of removing nutrients from pond effluent. Suspended solids, total phosphorus content, ammonia-nitrogen, and nitrate-nitrogen are efficiently removed in microcosms planted with Avicennia marina, Rhizophora stylosa, and Lumnitzera racemose (Su *et al*., 2019). Nutrient removal in ponds with mangrove seedlings is higher than in ponds without seedlings, thus improving water quality and reducing nutrients in the effluent (Moroyoqui-Rojo *et al*., 2012). In this study, only ammonia was removed from shrimp pond effluent drained into an area with mangrove at MPR=2:1. At higher MPR of 4:1, other parameters such as phosphate, TSS and chlorophyll a were also removed. This observation is in consonance with previous reports that MPR affects the efficiency of mangroves to remove nutrients from pond effluents. Reported MPR required to remove

Table 3. Levels of ammonia, phosphate, total suspended solids (TSS) and chlorophyll a observed in the receiving environment with, MPR=2:1, and without mangroves (Experiment 1). Values in the same column with the same superscript are not significantly different (P>0.05)

Day	Ammonia (ppm)		Phosphate (ppm)		TSS (ppm)		Chlorophyll a (ppm)	
	With mangroves	Without Mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves
0	.09^{c}	.09^{b}	.035^{c}	.01^{a}	355^{bc}	355	nm	nm
1	0.17^{d}	.17^{e}	.035^{c}	.025^{b}	455^{d}	340	nm	nm
2	nm	nm	Nm	nm	nm	nm	nm	nm
3	.00^{a}	.05^{a}	.01^{ab}	.02^{b}	375^{cd}	344	nm	nm
4	.00^{a}	.12^{cd}	.02^{bc}	.01^{a}	nm	nm	nm	nm
5	.00^{a}	.095^{bc}	.00^{a}	.01^{a}	246^{a}	304	nm	nm
6	.045^{ab}	.19^{e}	.055^{c}	.06^{ac}	nm	nm	39	39
7	.00^{a}	.08^{b}	.02^{bc}	.01^{a}	282^{ab}	348	38	44
14	.01^{ab}	.14^{d}	.02^{bc}	.02^{b}	340^{bc}	359	37	38
21	nm	nm	Nm	nm	nm	nm	nm	nm
28	nm	nm	Nm	nm	nm	nm	nm	nm

nm= not measured

Table 4. Mean ammonia, phosphate, total suspended solids (TSS) and chlorophyll a level observed in the receiving environment with (MPR=2:1) and without mangroves (MPR=0) (Experiment 1)

Parameter	Unit	With mangrove	Without mangrove	P-value
Ammonia-N	ppm	0.035	0.043	0.002
Phosphate-P	ppm	0.019	0.018	0.423
TSS	ppm	255.38	256.25	0.925
Chlorophyll a	ppm	37.71	40.85	<0.000

Table 5. Levels of ammonia, phosphate, total suspended solids (TSS) and chlorophyll a observed in the receiving environment with, MPR=4:1, and without mangroves (Experiment 2). Values in the same column with the same superscript are not significantly different (P>0.05)

Day	Ammonia (ppm)		Phosphate (ppm)		TSS (ppm)		Chlorophyll a (ppm)	
	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves
0	.08^{d}	.08^{b}	.085^{cd}	.085^{e}	118^{e}	118^{cd}	118^{e}	118^{e}
1	.035^{bcd}	.89^{i}	.085^{cd}	.055^{abcd}	34^{ed}	46^{b}	90^{d}	101^{d}
2	.06^{cde}	.36^{g}	.095^{d}	.055^{cde}	160^{b}	147^{f}	nm	nm
3	.045^{bc}	.25^{e}	.075^{cd}	.055^{cde}	31^{c}	43^{g}	nm	nm
4	.07de	.23^{e}	.115^{d}	.015^{abc}	nm	nm	nm	nm
5	.225^{f}	.165^{d}	.00^{a}	.006^{a}	110^{a}	165^{c}	nm	nm
6	.195^{f}	.13^{c}	.045^{bc}	.011^{ab}	nm	nm	nm	nm
7	.15^{e}	.405^{g}	.00^{a}	.05^{bcde}	14^{a}	111^{c}	33^{a}	68^{b}
14	.045^{bc}	.53^{h}	.03^{ab}	.07de	82^{cd}	123^{d}	74^{c}	76^{c}
21	.01^{a}	.055^{b}	.015^{ab}	.00^{a}	16^{a}	17^{a}	49^{b}	61^{ab}
28	.03^{ab}	.015^{a}	.00^{a}	.00^{a}	12^{a}	138^{e}	43^{b}	58^{a}

nm= not measured

Table 6. Mean ammonia, phosphate, total suspended solids (TSS) and chlorophyll a level observed in the receiving environment with (MPR=4:1) and without mangroves (MPR=0) (Experiment 2)

Parameter	Unit	With mangroves	Without mangroves	P-value
Ammonia-N	ppm	0.087	0.283	<0.000
Phosphate-P	ppm	0.045	0.036	0.176
TSS	ppm	64	100	<0.000
Chlorophyll a	ppm	67.94	80.31	<0.000

Table 7. Mangrove community structure of the mangrove area receiving shrimp pond effluent

Sampling Period	Trees (number/ha)	Saplings (number/ha)	Seedlings (number/ha)
A	980	1040	1850
B	1185	1333	2444
C	1466	1570	2963
D	1833	1800	3585

A= before the start of Experiment 2 Trial 1

B= before the start of Experiment 2 Trial 2

C= before the start of Experiment 2 Trial 3

D= one month after Experiment 2 Trial 3

Table 8. Average monthly increase in mangrove growth observed in mangrove habitat receiving and not receiving shrimp farm effluent over a 3-month period

	Average monthly increase in growth (mm)		
	Trees	Saplings	Seedlings
Receiving	5	12	52
Not Receiving	4	9	66

nutrients from pond effluent varies and has a wide range. An MPR of 2 to 89.9:1 is required to filter the phosphorus load (Robertson and Philipps, 1995; Shimoda *et al*., 2005); while an MPR of 1.8 to 5.4: 1 is required to remove nitrogen (Shimoda *et al*., 2007; Primavera *et al*., 2007) from farm effluents. The variability in the MPR required to remove nutrients from pond effluent could be attributed to the mangrove hydrodynamics (Robertson and Philipps, 1995). However, MPR of 4:1 reported to establish a healthy ecosystem (Saenger *et al*., 1983) can be implemented or used as rule of thumb. This is supported by the result of this study wherein NH4+, PO43-, TSS, and chlorophyll a in shrimp farm effluent were efficiently reduced in MPR=4:1.

As long as there is mangrove in the receiving environment or MPR is not zero, MPR does not seem to affect nitrogen removal. Nitrogen is removed from the pond effluent through nitrification and denitrification processes during which nitrogen is released into the environment making it available for plant growth (Dai *et al*., 2021; Wang *et al*., 2019). Bacteria responsible for the two processes, nitrification and denitrification, are abundant in mangrove habitats (Dai *et al*., 2021).

Phosphate is removed from the pond effluent by plant uptake and by the incorporation of phosphate into TSS (Ouyang and Guo, 2016; Ruzhitskaya and Gogina, 2017). The latter explains for the increase in the TSS level in receiving environments without mangrove; and the non-significant difference in the phosphate level in pond effluent drained in both types of receiving environments and in both MPR. Phosphate in the pond effluent bonded with the TSS in all types of environments.

Mangroves strip pond effluent of nutrients through plant assimilation. This is evident in this study by the increasing number of trees, saplings and seedlings after each experiment. Furthermore, this is also shown in the greater increase in plant growth in environment receiving shrimp farm effluents compared to those not receiving, except for the seedlings. The increase in the number and growth of mangroves in receiving environment with mangrove is consistent with the findings of Capdeville *et al*., (2019). Some of the seedlings being monitored for growth in the environment with mangroves also died. The lesser increase in growth and mortality observed in the seedlings in receiving environment with mangroves could be due to eutrophication. The increase in nutrient resulted in the increase in the number of seedlings that resulted in an overcrowding and eventually some of the older seedlings died and the others did not grow well.

In summary, the study demonstrated the efficiency of mangrove forest to remove nutrients, TSS and chlorophyll a from shrimp pond effluents. Although the levels of ammonia, phosphate, chlorophyll a and TSS were fluctuating, they are statistically lower in effluents drained into the mangrove habitat (MPR=2:1 and MPR=4:1) compared to area without mangroves. At MPR=4:1, ammonia is removed from the water after 3 days; TSS after 2 days; phosphate and chlorophyll after 7 days. At MPR=2:1, only ammonia can be efficiently removed and after 3 days. Mangroves purify the shrimp farm effluent by nutrient uptake as shown in the greater increase in stem length in saplings and trees in environments receiving the effluent.

Acknowledgement

The study was funded by the Government of Japan under the Trust Fund (GOJ TF 6) granted to SEAFDEC/AQD under study code FS-04-Y2015T.

References

Belak J, Dhar AK, Primavera JH, dela Peña LD, Pettit P, Alcivar-Warren A. 1999. Prevalence of viral diseases (IHHNV and WSSV) in *Penaeus monodon* from the Philippines and its association with mangrove status and shrimp culture systems. In: Alcivar-Warren A. (Ed.), Proceedings of the Aquaculture and Conservation of Marine Shrimp Biodiversity Symposium, Tufts University School of Veterinary Medicine, December 10, 1999.

Capdeville C., Abdallah K., Walcker R., Rols J.L., Fromard F., Leflaive J. 2019. Contrasted resistance and resilience of two mangrove forests after exposure to long-term and short-term anthropic disturbances. Marine Environmental Research 146: 12-23.

Dai Y, Lin X, Luo Y, Sun J, Tian Y. 2021. Molecular analysis of microbial nitrogen transformation and removal potential in mangrove wetlands under anthropogenic nitrogen input. Science of The Total Environment 773, 145632,

Donato DC, Kauffman JB, Kurnianto S, Stidham M, Murdiyarso D. 2011. Mangroves among the most carbon-rich forests in the tropics. Nature Geoscience 4: 293-297.

Fitzgerald WJ. 1997. Silvofisheries- an environmentally sensitive integrated mangrove forest and aquaculture system. Aquaculture Asia 2 (3): 9-17.

Gatune C, Vanreusel A, Cnudde C, Ruwa R, Bossier P, DeTroch M. 2012. Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae. Journal of Experimental Marine Biology and Ecology 426–427: 28–38.

Krebs CJ. 1999. Ecological methodology. Second Edition. Benjamin Cummings, Menlo Park, 620 p.

Le BT. 2006. The relationship between mangrove and the environment, and its effects on shrimp and mangrove productivity in the integrated shrimp-mangrove system in Ngoc Hien districts, Ca Mau province. In: Hong PN (ed.) The Role of Mangrove and Coral Reefs Ecosystems in Natural Disaster Mitigation and Coastal Life Improvement, pp. 145–155. IUCN, MERD, Hanoi, Viet Nam.

Lebata-Ramos MJH, Solis EFD, Sibonga RC, Watanabe S. 2012. Co-culture trials of sandfish Holothuria scabra and black tiger shrimp *Penaeus monodon* in mangroves. In: Tanaka K, Morioka S, Watanabe S (eds.). Sustainable Stock Management and Development of Aquaculture Technology Suitable for Southeast Asia. Tsukuba, Japan: Japan International Research Center for Agricultural Sciences; JIRCAS Working Paper 75; p. 87-95.

Moroyoqui-Rojo L, Flores-Verdugo FJ, Hernández-Carmona G, Casas-Valdez M, Cervantes-Duarte R, Nava-Sánchez EH. 2012. Nutrient removal using two species of mangrove (Rhizophora mangle and Laguncularia racemosa) in experimental shrimp (*Litopenaeus vannamei*) culture ponds. Ciencias Marinas 38(2): 333–346

Ouyanga X, Guo F. 2016. Paradigms of mangroves in treatment of anthropogenic wastewater pollution. Science of The Total Environment 544: 971-979.

Primavera JH, Altamirano JP, Lebata MJHL, Delos Reyes Jr AA, Pitogo CL. 2007. Mangroves and shrimp pond culture effluents in Aklan, Panay Is., central Philippines. Bulletin of Marine Science 80 (3): 795-804.

Rivera-Monroy VH, Torres LA, Bahamon N, Newmark,F, Twilley RR. 1999. The potential use of mangrove forests as nitrogen sinks of shrimp aquaculture pond effluents: the role of denitrification. Journal of the World Aquaculture Society 30: 12-25.

Robertson AI, Phillips MJ. 1995. Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. Hydrobiologia 295, 311–321.

Ruzhitskaya O, Gogina E. 2017. Methods for Removing of Phosphates from Wastewater. MATEC Web of Conferences 106, 07006.

Saenger P, Heger EJ, Davie JDS. 1983. Global Status of Mangrove Ecosystems. (Suppl.) Environmentalist 3: 1–88.

Shimoda T, Fujioka Y, Srithong C, Aryuthaka C. 2005. Phosphorus budget in shrimp aquaculture pond with mangrove enclosure and aquaculture performance. Fisheries Science 71:1249- 1255.

Shimoda T, Fujioka Y, Srithong C, Aryuthaka C. 2007. Effect of water exchange with mangrove enclosures based on nitrogen budget in *Penaeus monodon* aquaculture ponds. Fisheries Science 73: 221-226.

Standard Methods for the Examination of Water and Wastewater. 2017. E.W. Rice, R.B. Baird, A.D. Eaton, (editors); American Public Health Association, American Water Works Association, Water Environment Federation ISBN: 9780875532875.

Sudheer NS, Philip R, Singh ISB. 2011. *In vivo* screening of mangrove plants for anti-WSSV activity in *Penaeus monodon*, and evaluation of Ceriops tangal as a potential source of antiviral molecules. Aquaculture 311: 36–41.

Tendencia EA, Bosma RH, Verreth JAJ. 2011. White spot syndrome virus (WSSV) risk factors associated with farming practices in polyculture and monoculture *P. monodon* farms in the Philippines. Aquaculture 311: 87-96;

Tendencia EA, Bosma RH, Primavera JH, Verreth JAJ. 2012. Effect of different mangrove-to-pond area ratios on influent water quality and WSSV occurrence in *Penaeus monodon* semi-intensive farms using the greenwater culture technique. Aquaculture 362-363: 72-79.

Tran NH, Yakupitiyage A. 2005. The effects of the decomposition of mangrove leaf litter on water quality, growth and survival of black tiger shrimp (*Penaeus monodon* Fabricius, 1798). Aquaculture 250: 700–712.

Wang F, Chen N, Yan J, Lin J, Guo W, Cheng P, Liu Q, Huang B, Tian Y. 2019. Major Processes Shaping Mangroves as Inorganic Nitrogen Sources or Sinks: Insights from a Multidisciplinary Study. Journal of Geophysical Research: Biogeosciences 124 (5): 1194-1208

Integrated Production of Abalone, *Haliotis asinina*, and Sandfish, *Holothuria scabra*, Through Community-Based Resource Enhancement (CBRE) in Molocaboc Island in Sagay Marine Reserve, Philippines

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Abstract

This study conducted participatory enhancement of abalone *Haliotis asinina* and sandfish *Holothuria scabra* stocks using hatchery-bred and reared seeds released in the shores of Molocaboc Island in multi-use buffer zone of Sagay Marine Reserve, Philippines. The Community-Based Resource Enhancement (CBRE) process, implemented continuously from 2006 to 2019, include social and biophysical preparation, formulation of fisheries management and governance strategies, release of hatchery-reared juveniles, monitoring and periodic assessment, and socioeconomic impact assessment.

CBRE was implemented through a tri-party collaboration involving fisherfolks, local government and research institutions. The abalone and sandfish enhancement procedures comprised of breeding in hatcheries, rearing of juveniles either in nursery facilities or net cages in coastal areas, and release in enhancement areas.

A total of 11,500 tagged abalone juveniles were released in 11 batches in a protected coralline site from 2011 to 2015. Monthly monitoring showed increase in abalone catch per unit effort (3 divers, 1 hour fishing) in the release site from 0–2 individuals during baseline in 2011 to up to 150 individuals until 2019, including spill-overs without tags. Meanwhile, 96,400 hatchery-bred and reared sandfish juveniles were stocked in floating netcages in 15 batches and later released in sea ranch where mean density increased significantly from 3 to 138 individuals/ha in 2015 to 2019. Gleaning should comply with locally instituted catch-size regulation to sustain the fishery.

Additional project activities include the construction and operationalization of a small-scale solar-powered hatchery on-site; freeze-drying trials of abalone meat to improve market reach; and initiatives to replicate the project in Lahuy Islands in Caramoan, Camarines Sur. Aquaculture, through seed production, therefore plays a key role in enhancement of threatened high-value species.
Resource enhancement benefitted the fisheries through participatory management and eventually provided spill-overs to supplement income of marginalized fishers.

Keywords: stock enhancement, abalone, sandfish, community-based resource enhancement, Philippines

Introduction

Fishing and gleaning in nearshore areas are often the last resort livelihood among the low-income households in coastal communities in the Philippines. Declining fish catch is commonly reported as a major problem in coastal villages (Muallil, *et al*., 2014; Anticamara and Go, 2016; Servonnat, *et al*., 2019). There is over-fishing in most coastal fisheries in the Southeast Asian Region due to poverty and increasing human population (Stobutzki, *et al*., 2006; Salayo, *et al*., 2008. For export commodities, increasing demand from importing countries drive some species to threatened status, and worst to depletion (Pauly, *et al*., 1998; Sumaila, *et al*., 2007; Gephart and Pace, 2015).

For these reasons, the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) and the Government of Japan Trust Fund (GOJ TF) initiated a program on Stock Enhancement of Threatened Species in Southeast Asia (SE Program) under the GOJ TF4 (2005–2009) to undertake studies on developing strategies for stock enhancement of high-value threatened species, such as abalone (*Haliotis asinina*) and sandfish (*Holothuria scabra*), that are economically beneficial for fishers in the Philippines and the Region. Through a SEAFDEC resolutions, AQD was mandated to implement the aquavculture-based component of the SE Program in view of its seed production technologies (Primavera, *et al*., 2005). Hence, the capability-building component of the Program was identified to be conducted in a fishing community in the Philippines.

Under the GOJ-TF4, the socioeconomics aspect of the capability-building component of the SE Program focused on the ex-ante analysis of impacts of stock enhancement through a baseline survey and social preparation towards an integrated production of tradeable species such as abalone and sandfish. Under TF5 (2010-2014), the project dealt with strategies for managing enhanced resources through governance, stock release and participatory monitoring. Finally, under TF6 (2015-2019), the project was tasked to focus on the community-based integrated production of abalone and sandfish though seed culture, sea ranching and stock enhancement of these threatened species.

All throughout, this socioeconomics project was implemented through a tri-party collaboration between the organized group of fisherfolks, its local government, and SEAFDEC/AQD.

This paper therefore presents the summary of results of the socioeconomics project under the abovementioned phases of the GOJTF program. The series of activities implemented under the project evolved to be called the Community-Based Resources Enhancement (CBRE) process. It presents the chronology of activities and outcomes with emphasis on the integrated production of abalone and sandfish through stakeholder participation in stock enhancement activities.

The enhancement activities using hatchery-bred and reared juveniles of threatened high-value species primarily aims to restore its fisheries to provide supplemental income to marginal fishers while maintaining the health of the intertidal and reef environment (Bell, *et al*., 2006). This CBRE process applied and developed in this socioeconomics project

aims to contribute to the improvement of governance of coastal resources in the Philippines and similar areas in Member Countries in Southeast Asia.

The study

This CBRE project was implemented with the community of fisherfolks in Barangay Molocaboc from 2006 to 2019. Barangay is a local term for village which is the smallest political unit in the Philippines. Molocaboc is located in the multi-use buffer zone of the Sagay Marine Reserve (SMR) under the jurisdiction of Sagay City in Negros Occidental province in central Philippines (**Figures 1a and 1b**). The SMR was promulgated in 1995 through Republic Act 9106 in order to protect and conserve the ecological, biological, scenic, scientific, and educational features of the area. The SMR is also covered by the National Integrated Protected Areas System (NIPAS) Act or the Republic Act 7586 of 1992 which aims to protect landscapes and seascapes in the Philippines. Abalones and sandfish are among the fishery resources in the SMR that have become overexploited and its fishery is threatened (Maliao *et al*. 2004; Salayo *et al*. 2016).

Molocaboc has 7,177 population who are mostly dependent on fishing for food and livelihood. Some fishers glean for high-value abalone and sandfish to supplement income from capture fishing but not for home consumption. Since the promulgation of the SMR which cover 32,000 ha water area, the no-take areas almost cover all of the fishing grounds, including the three reef areas, namely Carbin (no-take core zone), Panal (partially protected) and Molocaboc (multi-use zone). This buffer area in the fringes of a no-take reserve is critical for the sustenance and livelihood of traditional fishing households in Molocaboc.

The fishing ground surrounding the three main groups of islands called Molocaboc Daku, Molocaboc Diut and Matabas comprise the multi-use area in the eastern portion of the SMR (**Figure 1b**). Thus, the release site for abalone juveniles was determined to be in the 4,000 m2 coral patches and rubbles located 2 km from the shore of Molocaboc Daku (**Figures 1c** and **1d**). The rearing area for sandfish is in the sandy seagrass area 1 km from the same shoreline (already mentioned under selection and assessment of release site, 3rd sentence). Aside from the social preparation activities prior to 2011, the floating signages and buoys in the release site inform the fisherfolks of the no-take regulation in the area (**Figure 1e**).

Methods

Social preparation

The project started with social preparation activities involving the representatives of the fisheries stakeholders. A tri-party collaboration between the fisherfolks of Molocaboc Island, local government of Sagay City, and researchers of SEAFDEC/AQD was formed. The terms of collaboration were stipulated in the Memorandum of Agreement signed by all parties. Social preparation involving baseline socioeconomic survey of 80 fisherfolks and validation of the survey results were conducted in 2007. Information, education and communication (IEC) campaigns through meetings, seminars, fairs and festivals were implemented as needed to address low level of awareness about stock enhancement and fisheries management. Fisherfolks were organized in 2009 to form the Barangay Molocaboc Fisheries and Aquatic Resources Management Council (FARMC) in adherence to Fisheries Administrative Order 196, series of 2000

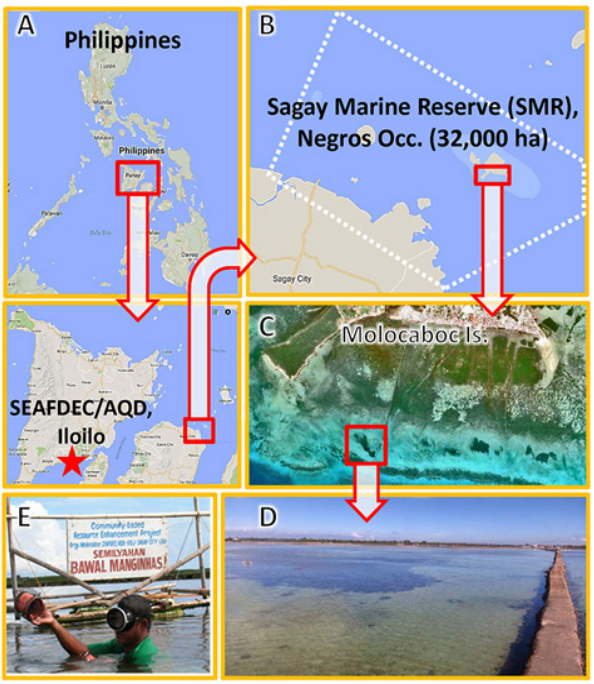

Figure 1. (A) Map of the Philippines showing the location of the study site in Negros Occidental (B) Coverage of Sagay Marine Reserve north of Sagay City; (C) Location of the Community-Based Resource Enhancement (CBRE) site on the southern shores of Molocaboc Island (source of 1A, 1B and 1C, Google Map); (D) Panoramic view of the CBRE site in coral area during low tide with exposed foot path leading to Molocaboc; (E) Signages in local dialect notify the public that "Gleaning is prohibited in this community-based breeding and nursery area"

of the Philippines' Bureau of Fisheries and Aquatic Resources (BFAR). The rules and regulations for managing enhanced stocks of abalone and sandfish were determined by the tri-party collaborators. The abalone fisheries in Molocaboc was determined to comply with the locally promulgated Barangay Ordinance in 2010 on catch size regulation to guide gathering, trade and consumption. Meanwhile, sandfish fisheries would have to comply with the nationally instituted BFAR Administrative Circular No. 248, series of 2013 on size regulation for sea cucumber collection and trade.

Selection and assessment of release site

The location of the release site for stock enhancement (10.9457 N°, 123.5590 E°) in Molocaboc Daku, one of the component islands of Barangay Molocaboc, was identified and an assessment of its suitability was done by all groups of stakeholders. The 4,000 m^2 release site for abalone juveniles is located in the coral area in the intertidal flats 2 kilometers from the shoreline alongside the 2 km × 1 m man-made cement structure where local island residents walk to take or alight from a boat during low tide. The rearing structures for sandfish such as floating hapa netcages, pens and sea ranch area were located in the sandy seagrass site adjacent to the abalone release site. Baseline sampling for the state of fisheries were determined from March to June 2011 for abalone, and in May 2015 for sandfish. Catch per unit effort (CPUE) for abalone was determined to refer to the number of individuals caught with bare hands by 3 local abalone divers in one-hour dive after an evening neap tide. Abalone divers use locally made foot

fins and guided by underwater flashlight. Sandfish density were determined by the number of individuals caught by hand per unit area gleaned during lowest tide following a neap tide.

Release and monitoring

The first release of diet-tagged hatchery-bred and reared (HR) abalone juveniles was done in June 2011 after the fourth and last month baseline sampling. Monthly monitoring after release of juveniles was conducted from July 2011 until August 2019, disrupted only when there is typhoon. Monitoring activities were conducted during evening neap tide when abalones are actively grazing for food carried by incoming tide and are out of the crevices of the corals where they inhabit. This avoids damages on the live corals and other substrates. The parameters monitored include CPUE and some morphometric parameters such as shell length (SL), body weight (BW), body mass index (BMI), sex and gonad stage of both the captured tagged hatchery-bred and reared abalones and the untagged individuals presumed to be those that recovered when the release site was protected against gleaners and those that are spill-overs. During these monthly monitoring activities, selected MOSRA (please spell out on first mention) members who are skilled abalone divers are involved in diving and measurement of morphometric parameters.

For sandfish, the stocking of early juveniles under this project was done from August 2015 to August 2019. Selected MOSRA members stocked and maintained the juveniles which were reared sequentially in floating hapa netcages setup in polyvinyl chloride frames, bamboo stake pens and open sea ranch areas. Fisherfolks worked with researchers in the monthly monitoring of survival, weight and length of sandfish. School children were sometimes involved in monitoring to improve awareness of stock enhancement and compliance to harvesting regulations. All fishing activities, including the gathering of abalone and sandfish, may continue outside of the no-take nursery and release site. However, fishers should comply with the >6 cm catch size regulation for abalone and the >320 g average live weight limit for sandfish

Results and discussion

Key activities and outcomes of the CBRE

The project started in mid-2006 through inception meetings within SEAFDEC/AQD to coordinate objectives and activities of different studies under the stock enhancement program. For this project on the capacity-building component of the stock enhancement program, the CBRE was implemented mainly through a tri-party collaboration of stock enhancement stakeholders. **Table 1** presents the chronology of key activities and corresponding objectives and results of each activity under the CBRE process. It also cites the literature that, in principle, guided these activities and objectives. The series of activities include:

1) social and biophysical preparation (establish stakeholder collaboration, baseline data collection and validation with stakeholders, information education and communication campaign, organization of stakeholders);

2) formulation of fisheries management and governance strategies (stakeholder capacity-building, consultative formulation of regulations for managing enhanced stocks);

3) release of hatchery-bred and reared

juveniles (site evaluation and selection, collection of broodstock and juvenile production, training on release strategies, participatory release;

4) monitoring and periodic assessment (determine practical parameters and success indicators); and

5) socioeconomic impact assessment (identify sustainable social, economic and environmental impacts).

Release of juveniles and monitoring results.

Figure 2 shows the CPUE during the monthly baseline sampling from February to June 2011 and during the monthly post-release monitoring from July 2011 to April 2019. The baseline sampling from March to June 2011 obtained 0-2 wild abalone individuals per unit effort. The low mean CPUE indicates that abalone used to exist but has become overfished in the release site. Considering the low CPUE, but with the availability of encrusting algae for food and branching corals Porites sp. for shelter, the site was selected for release of diet-tagged HB abalone juveniles that were produced and reared in SEAFDEC/AQD hatchery and nursery facilities. A total of 11,500 juveniles were released in 11 batches from June 2011 to April 2015. Release volumes and period were determined and limited by the availability of juveniles from the broodstock sourced from Panal Reef in SMR and brought to the SEAFDEC/AQD hatchery in Tigbauan, Iloilo. Monthly monitoring showed CPUE in the release site increased from 0-2 individuals during baseline sampling in 2011 to >150 individuals starting in 2017. From 2011 to 2013, most samples were HR individuals. Thereafter until 2019, HR samples declined until majority (98-100%) were without tags or presumed spill-overs. Other fishers were never prevented from gleaning outside the release site and complied to the locally instituted >6 cm shell length catch-size regulation. Regulated harvesting in the release site started 2 years after first release and continued to sustain funds for CBRE operations.

Figure 3 shows the results of participatory monitoring of HR sandfish juveniles released in 15 batches from December 2015 to September 2019. A total of 96,400 individuals of <0.01 g size was stocked in floating hapa net cages; of which 15% reached > 3g and released nursery pens; of which 13% were recovered and released in sea ranch at >20 g size. **Figure 3-B** shows the schedule of sandfish releases (blue bars) into the ranch. The number of animals released were dependent on the how many individuals grew >20g in the nursery pens every month. Overall, the mean density in sea ranch increased from 3 individuals/ha in 2015 to 138 individuals/ha in 2019, suggesting that the site was environmentally conducive for sandfish ranching. Meanwhile, **Figure 3-A** shows the profile of recovered sandfish during monthly monitoring with a reference line (red dotted line) at 320 g as the ideal minimum harvestable size. In as early as Feb 2017, a few harvestable individuals (black diamonds) already exceeded this line, while some much bigger (>500 g) individuals were even recorded in as early as May 2017. Although, some sandfish were already large enough to harvest, fisherfolk members were still adamant in collecting them for sale.

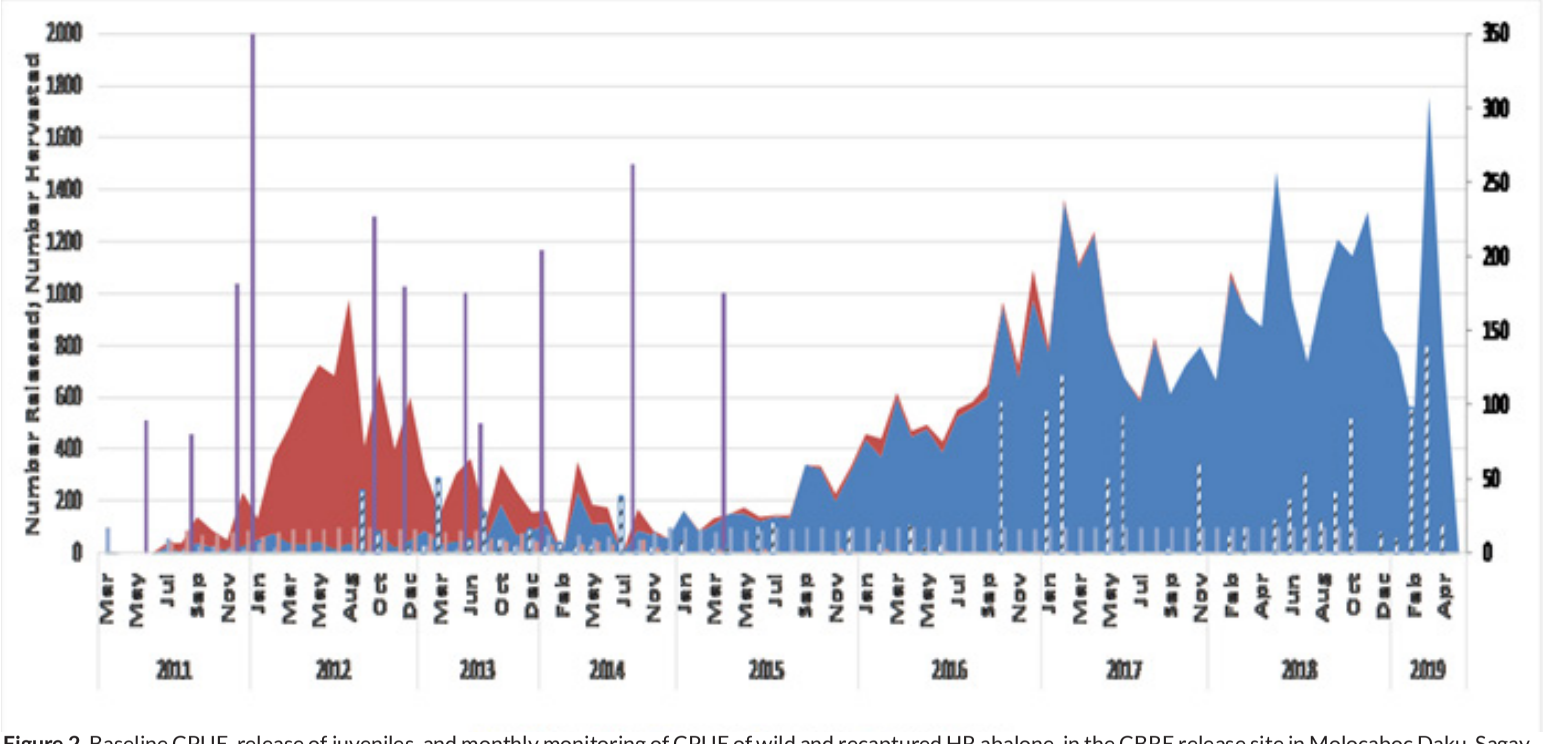

Figure 2. Baseline CPUE, release of juveniles, and monthly monitoring of CPUE of wild and recaptured HR abalone, in the CBRE release site in Molocaboc Daku, Sagay Marine Reserve, Negros Occidental, Philippines, from March 2011 to April 2019. (the cumulative or total number of released or harvested wild and HR abalones (light green color) is not visible in the figure; perhaps no need to have the text and figures in bold to make them more visible)

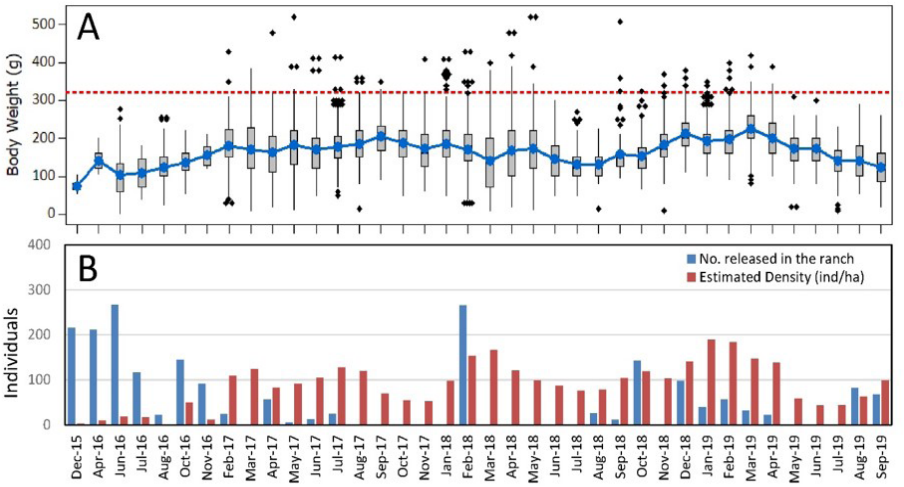

Figure 3. Sandfish population profile at the Molocaboc Dacu Sea Ranch from Dec 2015 to Sept 2019, showing (A) the box plot size profile of recovered sandfish (gray boxes indicating 25 % and 75 % percentile; whiskers lines as upper and lower limits; Blue dots connected by blue line represent the Mean; black diamonds are individual sandfish that are in the lowest and highest extremes), and (B) the number of sandfish released (sizes of >20g, blue bars) into the ranch with estimated density of recovered sandfish (red bars)

Participation of fisherfolks

The fisherfolk members of the BFARMC, later known as MOSRA, primarily protected the CBRE project site by guarding the area day and night against encroachment and destruction of the enhancement site. **Figure 4** shows an overall fluctuating and slightly declining trend in the number of man-days per month volunteered by fisherfolks in guarding the site. Initially, participation was low at around 20 man-days in 2011, suggesting that there were about 10 days in a month when no one is guarding the site. Participation increased in some months in 2012 and 2013 when there are more than 30 man-days guarding in a month or more than 1 person guarded the release site on some days and nights. But it continuously declined gradually until 2019 with less than 20 man-days per month dedicated in guarding the site. Sustaining voluntary participation is most often variable and a constraint in community-based fisheries undertakings. Often, reasons for non-participation in guarding the site, especially in the evening, are prioritization of fishing activities to obtain cash income, typhoon and cold months from October to January.

Complementary activities

During the course of monthly participatory monitoring of abalone and sandfish in the enhancement site, other activities were periodically conducted such as:

1) organizational meetings with other stakeholders, including local traders, school children and parents;

2) annual election of MOSRA officers; and

3) periodic harvesting and selling of harvest generate funds for MOSRA as a project exit strategy.

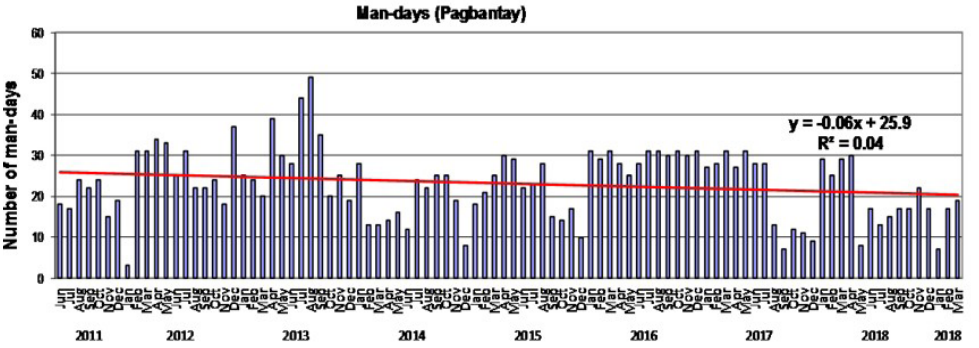

Figure 4. Number of man-days per month spent by fisherfolk-members in protecting the CBRE project site, Molocaboc, Sagay Marine Reserve, Negros Occidental, Philippines, from June 2011 to March 2019. Note: When man-days per month is greater than 30, it suggests that more than 1 person guarded the release site in a day and night

Table 1. Highlights of CBRE project activities and results from July 2006 to December 2019, Sagay City, Philippines

Year	Activities and Objectives	Result Highlights
2006	Project inception and collaboration agreement (Agbayani *et al.*, 2000)	Site visit and project briefing between collaborators such as Sagay City local government at the barangay and city levels, Molocaboc fisherfolks and SEAFDEC/AQD researchers.
2007	Survey of fishery stakeholders to gather baseline data (Bell *et al.*, 2006)	Survey revealed low-level of awareness of stock enhancement (17% of respondents). Key informant discussions with local government stakeholders showed willingness to collaborate towards CBRE in barangay Molocaboc.
2008	Stock enhancement information dissemination and campaign (Capinpin *et al.*, 1998; Bell *et al.*, 2006; Okuzawa *et al.*, 2006)	Conducted on-site seminar series on biology and life cycle of threatened abalone and sandfish, and opportunities for stock enhancement and other aquaculture technologies
2009	Organization and capacity-building of fisherfolks and other stakeholders (Jentoft 1989; Pomeroy *et al.*, 2001; Mahon *et al.*, 2008)	Fisherfolks organized as Barangay Molocaboc Fisheries and Aquatic Resources Management Council (BFARMC) compliant with FAO 960 of the BFAR and with guidance from PAMB-SMR.
2010	Participatory formulation of regulations for the management of enhanced stocks (Tringali *et al.*, 2008)	Promulgation and implementation of the Barangay Molocaboc Ordinance 01-Series of June 2010 on the gathering, harvesting, trade and consumption of abalone, with emphasis on the >6 cm catch size regulation and live local trade of abalone.

Year	Activities and Objectives	Result Highlights
2011	Baseline assessment of release site, stock release and monitoring (Gallardo *et al*., 2003; Prince 2004; Lebata-Ramos *et al*., 2013)	Baseline assessment of the release site showed its overfished status with CPUE at 0-2 abalone individuals. Diet-tagged 2.4 cm mean size hatchery-reared abalone juveniles were released periodically in no-take demarcated site; and sandfish juveniles were stocked in floating nets in the intertidal flats located 2 km from the shoreline of Molocaboc island. Fisherfolks guard and protect the released stocks with logistics support from the LGU.
2012	Strengthen governance of released stocks (Nasuchon and Charles, 2010)	The abalone catch size regulation promulgated at the barangay level in 2010 was upscaled by the Protected Area Management Board of SMR to be a Sagay City-wide regulation.
2013	Establish regulated harvest protocol for abalone	CPUE during monthly sampling showed increasing proportion of >6 cm harvestable sizes, hence harvesting protocol and standards were determined and practiced.
2014	Mid-project socioeconomic impact assessment (Lorenzen *et al*., 2013)	Survey of fisherfolks and traders showed improved understanding and support for stock enhancement. Local government renewed and enhanced support to CBRE project.
2015	Re-establish sandfish nursery and sea ranch activities, continue monitoring of abalone to follow through spill-overs	Survey of suitable sandfish sea ranch sites conducted with fisherfolks. Stocking of sandfish juveniles resumed and actively engaged fisherfolks in rearing and monitoring in cages, pens and sea ranch. Eleventh and final release of abalone juveniles on April 2015 but continued monthly monitoring until 2019 to evaluate performance of the released stocks together with recovered wild stocks and spill-overs.
2016	Continuous monitoring of sandfish sea ranch and regulated harvesting of abalone	Determined sustainable regulated harvest protocol and standards to generate funds for organization and operations. Re-organization of BFARMC into Molocaboc Sea Ranchers Association (MOSRA).
2017	Reinforcing fisherfolk entrepreneurship and replication of CBRE sites for abalone and sandfish	Participated in sustainable seafood campaign of high-end hotels to develop entrepreneurial skills of MOSRA fisherfolks. Initiated CBRE replicates in Molocaboc Diut and Lahuy Islands in Camarines Sur in collaboration with Partido State University, local government of Caramoan and fisherfolks
2018	Sustaining stock enhancement through seed production start-up in solar-powered hatchery	Zero recovery of tagged HR abalone and all catches are untagged individuals during monitoring from March 2018 onwards. Hatchery constructed and training on operations initiated. Freeze-drying trials to explore abalone product forms conducted but results need improvement.

Year	Activities and Objectives	Result Highlights
2019	Project impact assessment and performance evaluation (Prince 2004; Perez-Rufaza *et al*., 2008; Lorenzen *et al*., 2013; Barclay *et al*., 2016)	Preliminary results of survey of 300 project stakeholders, including abalone and sandfish gleaners and traders, MOSRA members and non-members and local government officers, indicate appreciation of the increased catch of abalone and sandfish that supplemented cash income of marginal fishers, and improvement awareness of stock enhancement as fisheries management strategy.

The project also implemented other activities as recommended during annual program reviews, such as construction and operationalization of a hatchery to train local fisherfolks in seed production to sustain stock enhancement.

The hatchery was solar-powered because there was no electricity supply in Molocaboc but pumping of sea water is supported by a petrol-powered generator. Initial hatchery runs need improvement in areas such as spawning frequency and performance, natural food production (i.e. *Navicula* sp. diatoms) and settling efficiency. Other tasks also involved initiation to replicate the CBRE together with other collaborators in similar suitable sites.

Conclusion and recommendations

The CBRE project in Molocaboc Island within the multi-use buffer zone of the SMR has overall achieved its objectives. Nursery culture of abalone and sandfish in the on-site hatchery were demonstrated to fisherfolks. Sea ranching of sandfish juveniles were done after advance nursery in floating hapa cages and rearing in pens in seagrass area; and stock enhancement of abalone juveniles were carried out in coral patches in the intertidal flats fronting the residential area of the island community.

Securing the ranching and enhancement sites from poachers are real challenges in poverty-stricken and overfished coastal areas. The day-to-night engagement of fisherfolks organized as Molocaboc Sea Ranchers Association (MOSRA), from being warden and co-managers together with the local government, enabled the rehabilitation of overfished abalone and sandfish population. Abalone CPUE in the release site and density of sandfish in sea ranch increased after release of juveniles.

The recovery of stocks can be attributed to:

1) socially prepared and responsive stakeholders, primarily the organized fisherfolks and the local government at the barangay and city levels;

2) formulation, implementation and compliance to bottom-up abalone fishery regulation, primarily the >6 cm shell length catch regulation for abalone, and some level awareness of the nationally instituted sea cucumber catch regulation arising from the information dissemination activities of this CBRE project;

3) availability of appropriate release protocol for hatchery-reared abalone and sandfish seed stocks spawned from remaining locally-sourced broodstock;

4) participation of fisherfolks, together with researchers, in monthly monitoring of the CPUE and morphometric growth parameters of abalone and sandfish;

5) logistical and governance support of the local government at the barangay and city levels;

6) compliance of stakeholders such as fishers, gleaners and traders to the catch size (>6 cm shell length for abalone) regulation instituted by the community and supervision of the fisherfolk association and the local government in implementing the ordinance; and

7) appreciation and local dissemination of the preliminary project outcomes witnessed by the participating stakeholders and beneficiaries such as gleaners whose catch increased due to spill-over from released stocks.

Aquaculture therefore plays a key role in the rehabilitation of threatened high-value species. Seed production technology and appropriate release strategies should be available to support any stock enhancement initiative. Research on breeding and nursery techniques for threatened aquatic species, especially the targeted economically important and tradeable commodities should be supported at the onset of indicators of overfishing of some resources.

Sea ranching and stock enhancement are high investment and long-term strategies to address depletion of fishery resources. However, these are sustainable strategies when collaboratively conducted with stakeholders who are willing to share their available resources categorized either as:

1) research funds from government and partner institutions;

2) logistics and governance support from local government; and 3) manpower and local knowledge of the resources among fisherfolk stakeholders. More so, resource enhancement projects do not only provide spill-overs to supplement the income of the most marginalized gleaners without fishing boats in fishing communities.

It also provided opportunities in terms of enriching the indigenous knowledge of fisherfolks with science-based enhancement procedures shared by researchers through participatory activities. Overall, the collaborative CBRE process applied in this project benefited the fishing environment. There was shared management of the resources between the relevant local government and the fisherfolks. The latter were eventually transformed from being resource users to sea ranchers.

Acknowledgements

The authors thank SEAFDEC/AQD and collaborators such as the Barangay Molocaboc FARMC/MOSRA and PAMB-SMR of Sagay City. Funds and program support were provided by the GOJTF (8100-T-RD-SE0105, SE0110 and SE0015). The authors acknowledge the inputs from GOJTF Co-Managers Dr Koichi Okuzawa, Dr Hiroshi Ogata, Dr Teruo Azuma, Dr Takuro Shibuno, Dr Chihaya Nakayasu and Dr Koh-ichiro Mori.

References

Agbayani, RF, Baticados, D.B. and Siar, S.V. 2000. Community fishery resources management on Malalison Island, Philippines: R&D Framework, interventions, and policy implications. Coastal Management, 28:19-27.

Anticamara, J.A. and Go, K.T.B. 2016. Spatio-temporal declines in Philippine fisheries and its implications to coastal municipal fishers' catch and income. Front. Mar. Sci., 3(21):1-10.

Barclay, K., Voyer, M., Mazur, N., Payne, A.M. Mauli, S., Kinch, J. Fabinyi, M. and Smith, G. 2017. The importance of qualitative social research for effective fisheries management. Fish. Res., 186: 426 - 438

Bell, J.D., Bartley, D.M., Lorenzen, K. and Loneragan, N.R. 2006. Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. Fish. Res. 80:1-8.

Capinpin, E.C., Encena, V.C. and Bayona, N.C. 1998. Studies on the reproductive biology of the donkey's ear abalone, Haliotis asinina (Linne.). Aquacult. 166, 141-150.

Gallardo, W.G., Bautista-Teruel, M.N., Fermin, A.C. and Marte, C.L. 2003. Shell marking by artificial feeding of the tropical abalone, Haliotis asinina (Linne.) juveniles for sea ranching and stock enhancement. Aquacult. Res., 34(10):839-842.

Gephart, J.A. and Pace, M.L. 2015. Structure and evolution of the global seafood trade network. Envi. Res. Let. 10(12), 125014, 2015.

Jentoft, S. 1989. Fisheries co-management: Delegating government responsibility to fishermen's organizations. Mar. Pol. 13(2):137-154.

Lebata-Ramos, M.J.H. Doyola-Solis, E.F.C., Abroguena, J.B.R., Ogata, H., Sumbing, J.S. and Sibonga, R.C. 2013. Evaluation of post-release behavior, recapture, and growth rates of hatchery-reared abalone Haliotis asinina released in Sagay Marine Reserve, Philippines, Rev. Fish. Sci. 21(3-4):433-440.

Lorenzen, K, Leber, K.M. and Blankenship, L. 2010. Responsible approach to marine stock enhancement: An update. Rev. Fish. Sci. 18(2):189-210.

Lorenzen, K., Agnalt, A.L., and Blankenship, H.L. 2013. Evolving context and maturing science: aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. Rev. Fish. Sci. 21(3-4):213-221.

Mahon, R., P. McConney, and R. Roy. 2008. Governing fisheries as complex adaptive systems. Mar. Pol. 32:104-112.

Maliao, R.J., Webb, E.L. and Jensesn, K.R. 2004. A survey of stock of donkey's ear abalone, Haliotis asinina L., in the Sagay Marine Reserve, Philippines: Evaluating the effectiveness of marine protected area enforcement. Fish. Res. 66:343-353.

Muallil, R.N., Mamauag, S.S., Cababaro, J.T., Arceo, H.O. and Aliño, P.M. 2014. Catch trends in Philippine small-scale fisheries over the last five decades: The fishers' perspectives. Mar. Pol. 47:110-117.

Nasuchon, N. and Charles, A. 2010. Community involvement in fisheries management: experiences in the Gulf of Thailand countries. Mar. Pol. 34(1):163-169.

Okuzawa, K., Lebata, J., Buen-Ursua, S.M. and Quinitio, E.T. 2005. The SEAFDEC/AQD Experience in Stock Enhancement. In. Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern. Southeast Asian Fisheries Development Center/ Aquaculture Department & Government of Japan Trust Fund, Iloilo City, Philippines. 150pp.

Pauly, D., Christensen, V. Dalsgaard, J. Froese, R. and Torres, E.C. Jr. 1998. Fishing down marine food webs. Science 279:860-863.

Perez-Rufaza, A., Rodriguez, E.M., Marcos, C., Zamarro, J.M., Stobart, B., Harmelin, M., Polti, S. Planes, S., Garcia-Charton, J.A. González-Wangüemert, M. 2008. Modelling spatial and temporal scales for spill-over and biomass exportation from MPAs and their potential for fisheries enhancement. Jour. Nat. Conserv. 16(4):234-255.

Pomeroy, R.S., Katon, B.M. and Harkes, I. 2001. Conditions affecting the success of fisheries co-management. Lessons from Asia. Mar. Pol. 25(3): 197-208.

Primavera, J.H., Quinitio, E.T. and Eguia, M.R.R. 2005. Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern, 13-15 July 2015. Southeast Asian Fisheries Development Center/ Aquaculture Department & Government of Japan Trust Fund, Iloilo City, Philippines. 150pp.

Prince, J.D. 2004. The decline of global abalone (genus Haliotis) production in the late twentieth century: Is there a future? In., Leber, K.M., Kitada, S. Blankenship, H.L. and Svåsand, T. (eds.) Stock enhancement and sea ranching: developments, pitfalls and opportunities. 2nd Edition, Blackwell Publishing Ltd. pp. 427-433.

Salayo, N.D., Garces, L., Pido, M., Viswanathan, K., Pomeroy, R., Ahmed, M., Siason, I., Seng, K. and Awae, M. 2008. Managing excess capacity in small-scale fisheries: Perspectives from stakeholders in three Southeast Asian countries. Mar. Pol. 32(4):692-700.

Salayo, N.D., Castel, R.J.G., Barrido, R.T., Tormon, D.H.M. and Azuma, T. 2016. Community-based stock enhancement of abalone Haliotis asinina in Sagay Marine Reserve: Achievements, limitations and directions. Pp.131-135. In: Hajime, K., Iwata, T., Theparoonrat, Y., Manajit, N., Sulit, V.T. (Eds.). Proceedings of the Symposium on Strategy for Fisheries Resources Enhancement in the Southeast Asian Region. Consolidating the Strategies for Fishery Resources Enhancement in Southeast Asia, Thailand (July 2015, SEAFDEC).

Servonnat, M., Kaye, R. Siringan, F.P., Munar, J. and Yap, Y.T. 2019. Imperatives for conservation in a threatened center of biodiversity. Coast. Manage. 47(5): 453-472.

Stobutzki, I.C., Silvestre, G.T. and Garces, L.R. 2006. Key issues in coastal fisheries in South and Southeast Asia, outcomes of regional initiative. Fish. Res., 78(2-3):109-118.

Sumaila, U.R., Khan, A., Watson, R., Munro, G., Zeller, D., Baron, N., and Pauly, D. 2007. The World Trade Organization and global fisheries sustainability. Fish. Res., 88(1-3), 1-4.

Tringali, M.D., Leber, K.M. Halstead, W.G., McMichael, R., O'Hop, J., Winner, B., Cody, R., Young, C., Neidig, C., Wolfe, H, Forstchen, A. and Barbieri, L. 2008. Marine stock enhancement in Florida: A multi-disciplinary, stakeholder-supported, accountability-based approach. Rev. Fish. Sci. 16(1-3):51-57.

Promotion of Resource Enhancement of Seahorse in an Island Community in Negros Occidental, Central Philippines

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Abstract

To promote the protection and sustainability of seahorses for their conservation, efforts have been done through stock enhancement by releasing captive-bred seahorses. However, preparatory activities are necessary for the development of long-term program on seahorse stock release and enhancement by conducting baseline stock assessment, developing the appropriate release and monitoring strategies, and encouraging the involvement of concerned communities in the management of the natural seahorse resources. The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD), with support from the Government of Japan Trust Fund (GOJ-TF), initiated seahorse resource enhancement efforts in a remote island community in Negros Occidental in central Philippines. Baseline assessment in 2012 to 2019 of the natural stock of seahorses showed an increasing number of stocks over the years. Results of the transport trials of juvenile seahorses (5-7 cm in stretched height (SH)) suggest an optimum stocking density of 3 ind/L for transport duration up to 12 hours. Appropriate protection of the natural habitat and with no gleaning of various intertidal species, the main source of income for the coastal community, suggests a possible sustainability of the wild seahorse stock. The community involvement may be promoted by active participation thru information, education and communication (IEC) and hands-on trainings during field sampling, seed production and nursery rearing of seahorses. Relevant information derived from the activities in the island community may serve as a model for the resource enhancement of seahorses in other potential sites in the Philippines and other countries in the region.

Introduction

Seahorses (*Hippocampus* spp.) are among the first marine fishes of commercial importance to be listed in the International Union for Conservation of Nature (IUCN) and Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES). They are considered vulnerable species due to habitat degradation and pressure on dwindling natural stocks arising from illegal, unreported and unregulated collection for traditional Chinese medicine and to a lower extent, the marine aquarium and curio trade (Vincent *et al*., 2011; Foster *et al*., 2016).

Resource enhancement efforts for seahorses have been initiated by the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) with support from the Government of Japan Trust Fund 5 (GOJ-TF5) through the project entitled "Resource enhancement of internationally threatened and over-exploited species in Southeast Asia through stock release." However, the release of captive-bred animals to augment the threatened wild populations needs careful groundwork to avoid negative impact on the wild seahorse populations resulting from disease introductions and genetic contamination of the natural population. The study aims to develop long-term program on seahorse stock release and enhancement by conducting baseline stock assessment, developing appropriate release and monitoring strategies, and encouraging the involvement of concerned communities in the management of the natural seahorse resources.

Methodology

Baseline assessment and monitoring of seahorse population

Baseline assessment of the natural stocks of seahorse was conducted in Molocaboc Island, a remote island community in Sagay Marine Reserve, Sagay City, Negros Occidental in central Philippines (**Figure1**) from October 2012 to December 2019. DNA samples were also collected for genetic analyses of wild and hatchery-reared seahorses to ensure the genetic integrity of the release program.

Monthly monitoring and collection of wild seahorses was conducted on a 12,000 m2 patch reef in Molocaboc Island. Prior to collection, the divers were trained on proper handling of live seahorses with the use of PVC pipes with net covers (**Figure 2**) as temporary holders for seahorses to minimize stress on the animals. Divers also received informal

Figure 1. Location of study site in Molocaboc Island, Negros Occidental, Central Philippines

lecture on seahorse biology and training on measurements of stretched height, body weight and examination of gonadal development stages. Four (4) local divers collected the seahorses for one hour during night time at the onset of high tide. The collected seahorses were individually measured for stretched height (SH) and weighed, and their gonad developmental stages classified (**Figure 3**).

Transport of seahorses from hatchery to release site

Experiments on the optimum stocking density, transport duration and acclimation of juvenile seahorses were conducted with three replicates each for three size groups: size A – 5 cm SH, size B – 6 cm SH and size C – 7 cm SH (Ursua, in prep). Seahorses were transported using styroboxes containing

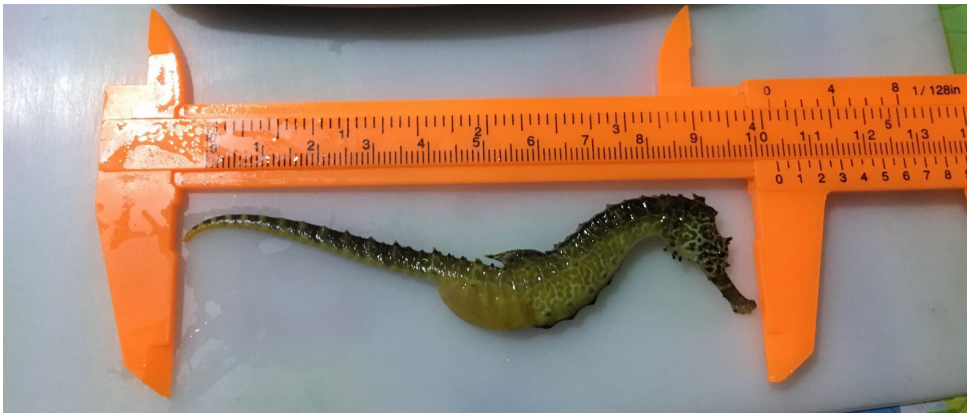

Figure 2. Measuring seahorse stretched height prior to transport trials

Figure 3. PVC pipes covered with nets as temporary holders for captured seahorses

well-oxygenated seawater. Nylon twines tied to lead sinkers were provided as holdfast for seahorses during transport. Five stocking densities (1, 2, 3, 4 and 5 ind/L) and two transport durations (10 and 12 hours) were tested.

At the release site, juvenile seahorses were stocked in intermediate enclosures made of B-nets (1 x 1 x 1 m^3) and cylindrical plastic screen cages (30 cm long, 25 cm diameter) hanged on a floating bamboo raft to observe post-transport survival of seahorses for 7 days.

Hatchery technique and fixed bottom nursery pens for seahorse in a remote coastal community

A simple hatchery facility was built for the production of juvenile seahorses in a remote coastal community in Molocaboc Island using the available natural food for seahorse in the area, optimum water exchange and solar powered mild aeration.

Broodstock were obtained by collecting pregnant male seahorse from the patch coral reef area and transferred to 10-L plastic pails at a stocking density of 1 ind/5 L. Maintenance of water includes daily siphoning of pail bottom at 30-50 % water change and mild aeration using solar-powered aerators. After parturition (giving birth), broodstock were released back in the coral reef.

Phototactic natural food organisms were collected at night using a plankton net. Copepods and mysids were separated using a sieve of 40 and 110 um plankton nets. Mysid shrimps were fed to adult and juvenile seahorses, while copepods were fed to newborn seahorses. Stocking density for the newborn and juvenile seahorses were 5 ind/L and 3 ind/L, respectively (**Figure 4**). Feeding was *ad libitum*.

Submerged fixed-bottom nursery pens were used for further rearing of 3 to 6 month-old hatchery-produced juvenile seahorses. Coral rubbles from the pilot site served as substrate for the juvenile seahorses.

Community involvement in resource enhancement of seahorses

Another activity to promote resource enhancement of seahorses in the community is through the distribution of information, education and communication (IEC) materials. Lectures were also provided among elementary and high school students on the biology and resource management of seahorses, including the baseline data of wild seahorse population collected in Molocaboc Island.

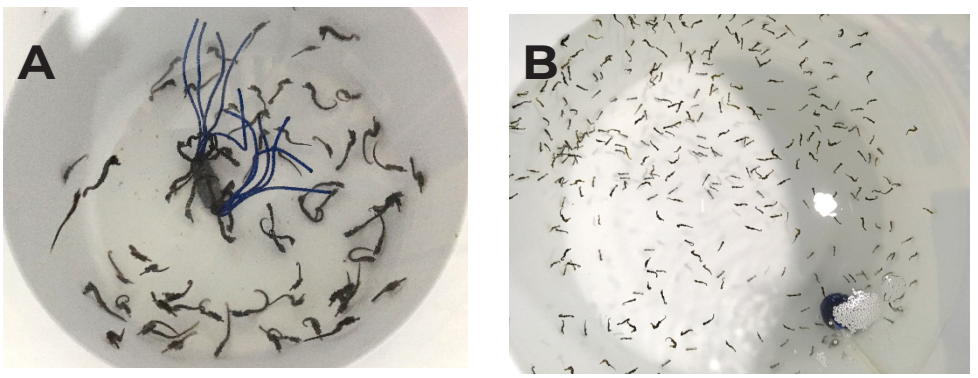

Figure 4. Newborn (A) and juvenile (B) seahorses reared in 10-L plastic pails

Results and discussion

Baseline assessment and monitoring of seahorse population

Mean seahorse SH measured 11.9-12 cm, while mean body weight ranged from 5.7 to 8.5 g. Partially and fully mature gonads of males and females were present year-round. The average number of seahorses (34 ind/sampling) in 2019 is higher than those observed from 2016 to 2018 (30 ind/sampling), in 2015 (18.7 ind/sampling) and from October 2012 to December 2013 (4.6 ind/sampling). The observed density (0.0028 m^{-2}) of seahorses in Molocaboc Island in 2019 is lower than the density (0.02 m^{-2}) of seahorses reported in Bohol Province, east -central Philippines (Martin-Smith *et al*., 2004).

Transport of seahorses from hatchery to release site

No mortality was observed between the size groups at different stocking densities and transport durations tested. However, following 7 days post-transport, survival of seahorses held at stocking densities of 4 and 5 ind/L, ranged from 70-85 % and 50-60 %, respectively. The transport trials on three size groups of juvenile seahorses (5, 6, and 7 cm SH) showed an optimum stocking density of 3 ind/L in all size groups at 10 and 12 h transport durations.

Hatchery technique and fixed bottom nursery pens for seahorse in a remote coastal community

Parturition of pregnant male seahorses usually occurs within 3 days after collection of broodstock. There was no record of mortality among seahorses which gave birth in the hatchery facility. The average survival rate of newborn seahorses ranged from 60-80 % after 30 days at stocking density of 5 ind/L. The juvenile seahorses have an average of survival rate of 40-60 % after 6 months at stocking density of 3 ind/L.

Juvenile seahorses reared in submerged fixed bottom nursery pens subsist on the available planktons in the water column. However, survival was variable due to the challenges encountered in monitoring of juveniles inside the pens. Rearing of seahorses in pens was labor intensive since it requires regular maintenance by cleaning the pens of algal assemblages and other fouling organisms.

Community involvement in resource enhancement of seahorses

Campaign drive on the management of natural resources highlighting seahorse biology and conservation was conducted annually in Molocaboc Island and attended by students, school teachers, fishermen organization members, and local government officials. On November 22, 2017 a total of twenty (20) students, 10 in the elementary and 10 in the secondary level of Molocaboc Integrated School participated in the Draw and Tell Contest with the theme "My role in the promotion of seahorse as a natural resource in my community." Their art works showed seahorse in corals and sea grasses, highlighting the importance of protecting the natural habitat of seahorses to protect the dwindling population of seahorses in the wild (**Figure 5**).

Conclusion and recommendation

The natural stock of seahorses in the pilot site showed an increasing number over the years. Appropriate protection and minimal disturbance of the natural habitat of seahorses suggests that the wild stock may recover and be sustainable. Monitoring of seahorse population should

Figure 5. Engaging students through lectures (left photo) and drawing activities (right photo) to raise awareness and promote conservation of seahorses in Molocaboc Island

be done periodically by the local divers. The community involvement may be promoted by active participation thru IEC and hands-on trainings during field sampling, seed production and nursery rearing of seahorses. Resource enhancement of seahorses may be promoted by learning from relevant information derived from the activities in the pilot site in Molocaboc Island and may serve as model for other potential sites in the Philippines and other countries in the region.

Acknowledgement

The author would like to thank Mariano Jarina, Joebert Nunez and Francisco Bascar for their support and assistance in Molocaboc Island; Ryan Q. Tigres for technical assistance; Drs. Nerissa D. Salayo, Jon P. Altamirano, Leobert D. de la Pena, Takuro Shibuno and Koh-Ichiro Mori for their constructive suggestions and advises; and SEAFDEC/AQD and GOJ-TF5 for funding support under study code 8300-T-RD-FS0215.

References

Foster, S. J., S. Wiswedel, and A. C. J. Vincent. 2016. Opportunities and challenges for analysis of wildlife trade using CITES data—seahorses as a case study. Aquat. Conserv.- Mar. Freshw. Ecosyst. 26: 154–172

Martin-Smith KM, Samoilys MA, Meeuwig JJ and Vincent ACJ. 2004. Collaborative development of management options for an artisanal fishery for seahorses in central Philippines. Ocean Coast. Manag. 47: 165-193

Ursua, SMA. Optimum stocking density, duration for the transport and acclimation of juvenile seahorse *Hippocampus* comes. In prep.

Vincent, A. C. J., S. J. Foster, and H. J. Koldewey. 2011. Conservation and management of seahorses and other Syngnathidae. J. Fish Biol., 78: 1681–1724

Vincent AC and Koldewey HJ. 2006. An uncertain future for seahorse aquaculture in conservation and economic contexts. ***In***: Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern, JH Primavera, ET Quinitio and MRR Eguia (eds). Aquaculture Department. Southeast Asian Fisheries Development Center (SEAFDEC/AQD), Tigbauan, Iloilo, Philippines; pp 71-84

Woods CMC and Martin-Smith KM. 2004. Visible implant fluorescent elastomer tagging of the big-bellied seahorse *Hippocampus abdominalis*. Fish. Res. 66: 363-371

Training Updates on Marine Fish Hatchery

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Abstract

One of the major constraints confronting the sustainable development of the aquaculture industry in the world, particularly in Southeast Asia, is the availability of quality fish seeds for stocking in ponds and cages. With the unreliable supply from the wild, the establishment of fish hatcheries has long been recognized as a primary means of reducing pressure on wild stocks and plays a key role in supplying the demand for seedstock of fish farmers.

SEAFDEC/AQD has been conducting a short-term training course on marine fish hatchery since 1985, in response to the growing aquaculture industry and the increasing demand for information on seed production of culturally important and high-value marine fishes such as milkfish, sea bass, rabbitfish, groupers, red snapper, and pompano.

The training conducted last June 19–25, 2018 was attended by participants from Myanmar, Indonesia, Philippines, Tanzania, and Maldives.

This 37-days training course aims to equip the participants, working in the government, academe as well as in the private sectors, with technical knowledge through lectures and practicals on fish broodstock management, spawning, larval rearing, natural food culture, fish health management, nursery and grow-out culture, special topics on hatchery design, recirculating system, among others, of milkfish (*Chanos chanos*), sea bass (*Lates calcarifer*), groupers (*Epinephelus coioides* and *E. fuscoguttatus*), mangrove red snapper (*Lutjanus argentimaculatus*), rabbitfish (*Siganus guttatus*) and pompano (*Trachinotus blochii*). The participants conducted monitoring, culture, and scaling up of natural food organisms (green algae, *Nannochlorum* sp. and brown algae, *Skeletonema tropicum*; sampling of fish broodstock for induced spawning, and larval rearing.

The trainees successfully cultured, monitored the growth rate, and scaled-up the production of natural food organisms (green algae, *Nannochlorum* sp. and rotifer, *Brachionus plicatilis*) for fish larvae. At the end of each larval rearing runs, the trainees obtained the following percent survival rate: milkfish (12-33); snapper (7-13); rabbitfish (5-19); pompano (0.4-6); sea bass (26-40); and grouper (22-31). The trainees cited poor water quality in the larval rearing tanks, especially at the onset of the rainy season, as one of the main reasons for the low survival rates.

Maintenance of good water quality during larval rearing is an important factor for the efficient operation of a fish hatchery. To ensure a sustainable fish seed production and high survival rates, critical water quality parameters should be monitored and maintained within optimum levels at all times.

The trainees evaluated the conduct of the course and gave an overall rating of 4.46 (very good).

Aquatic Animal Health

The Status of Aquatic Animal Health in Cambodia

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Abstract

Human population in Cambodia keeps increasing from year to year and the demand for food consumption also increases. Food products in Cambodia come from two main sources: terrestrial and aquatic. In this sense, aquaculture has been playing very important roles to produce aquatic food products in order to provide the sustainability of national food security, economy and also minimize the pressure on Cambodia's capture fisheries. Aquaculture production in Cambodia has grown by an average of 20 % per year over the past decade, increasing from less than 50,000 metric tons in 2008 to 207,443 metric tons in 2017. However, disease is considered as the most serious problem that can limit the aquaculture production in many countries in the world including Cambodia. Previous reports showed that in 1999 intensive shrimp farming systems in Cambodia were severely affected by White Spot Syndrome Virus (WSSV), Monodon Baculovirus (MBV) and Yellow Head Virus (YHV). Hence, shrimp farming areas dropped from 1,000 hectares to 850 hectares in the year 2000 and gradually decreased each year. WSSV is the most serious threat faced by the shrimp farmers in Cambodia and is probably the major cause of direct losses of up to $ 14.5 million per year. During 2011–2013, white leg shrimp (*Litopenaeus vannamei*) was seriously infected by Early Mortality Syndrome (EMS) in Koh Kong Province. To date, extensive and semi-intensive shrimp farming have started mainly in Kampot, Kep and Preah Sihanouk and Koh Kong. Only one super intensive RAS Indoor white leg shrimp farm in Kampot operated in 2019.

Recently, fish health monitoring of freshwater fish farms in 10 provinces and sea bass farms in three coastal provinces were conducted by the cooperation of central officers, officers from Marine Aquaculture Research and Development Center (MARDeC) and provincial officers. Fish samples were diagnosed and analyzed in Aquatic Animal Health Laboratory of MARDeC. The results showed that several of fish pathogens were identified such as fish parasites: *Trichodina* sp., *Ichthyophthirius multifiliis*, *Epistylis*, *Apisoma*, *Dactylogyrus* sp., *Gyrodactylus* sp., *Argulus* sp., *Acanthocephalan*, *Henneguya* sp., *Cryptocaryon irritans*, *Caligus* sp., *Lernaea* sp., *Benedinia* sp., *Ancyrocephalidae* sp., *Amyloodinium* sp. and *Myxozoa* sp. Pathogenic bacteria: *Aeromonas* spp., *Edwardsiella ictaluri*. Fungi: *Aphanomyces invadans* and *Saprolegnia* sp. Diagnostic laboratories can perform level I and II but not for all species of aquatic animals and diseases; level III is not yet effectively performed due to the lack of facilities, skills and knowledge.

Keywords: aquatic animal health, diseases outbreak, aquaculture, Cambodia

Introduction

Cambodia, located in the continent of Asia, covers 176,515 square kilometers of land and 4,520 of water, making it the 90th largest nation in the world with total area of 181,035 square kilometers. Bordered by Thailand and Laos to the northwest, Viet Nam to the east and the Gulf of Thailand to the Southwest. Cambodia has a population of over 16 million. There are many natural water sources in Cambodia like the Tonle Sap or Tonle Sap Lake, the biggest lake in the country and situated in the central part of the country. The lake's size varies considerably over the year from an area of around 2,500 square kilometers at the end of dry season in late April to an area of up to 16,000 square kilometers during the rainy season in September to early October. The lake was one of the most abundant inland waters in the world where floodplains and shrubs provide the habitat and breeding grounds for fish and other aquatic animals. It was reported that more than 500 freshwater fish species (Rainboth, 1996) inhabit the lake. On the other hand, more than 562 marine fish species were identified (Try, 2003; Hortle, 2007) in Cambodia's coastal zone located on the Southwest of the country extending 435 km in the gulf of Thailand (Hav and Leap, 2005). Cambodians consume 52 kg of fish per person per year which was recorded as one of the highest level of fish consumption in the world. Remarkably, people who lived around Tonle Sap Lake consumed fish between 67–80 kg per capita per year (Lang, 2015).

Human population in Cambodia keeps growing every year and the demand for food also increases (Joffre *et al*., 2019). Moreover, abrupt climate changes, destruction of floodplain for agricultural lands, hydropower dam constructions in upstream Mekong River, and some illegal fishing are considered as the major challenges causing the fluctuation of Cambodia's capture fisheries (Khan *et al*., 2019). Aquaculture is an alternative way to minimize the pressure on Cambodia's capture fisheries and has potentially contributed to national food security, economic sustainability and promote poverty alleviation (SPF, 2015).

Seed production rapidly increased from less than 20 million in early 2000s to approximately 180 million in 2015. Fifty-five percent (55 %) of the seeds are imported from adjacent countries, 13 % derived from the wild and 32 % from Cambodian state and private hatcheries. Freshwater cage culture has been practiced for many centuries in Great Lake, Tonle Sap and the Upper Mekong river. Cage culture contributed about 50 % to total aquaculture production with several main species including giant snakehead (*Channa micropeltes*, 47 %), pangasius (*Pangasianodon hypophthalmus*, 27 %), and hybrid catfish (*Clarias*, 27 %) and other species (3 %). Marine and brackish water aquaculture are relatively under developed. Marine cage culture began in the late 80s and early 90s in Kampot and Koh Kong provinces (NSPAD, 2017). It was mostly practiced in small scale farms in Preah Sihanouk, Kampot, Kep, and Koh Kong provinces located along the Cambodian coastal line. Farming was mainly based on seeds of sea bass, grouper, cobia, and pompano that were produced either from governmental hatcheries, from the wild, or were imported (Sorphea *et al*., 2018). According to official statistics of Fisheries Administration, aquaculture production increases by an average of 20 % per year over the past decade, increasing from less than 50,000 metric tons in 2008 to 207,443 metric tons in 2017. Aquaculture accounts for 20 % of the total fish production in the country and

the majority of aquaculture production is from inland aquaculture that accounts for nearly 90 % (Joffre *et al*., 2019). Aquaculture in Cambodia has been quickly increasing year by year as mentioned, but disease outbreaks are the main constraint in developing aquaculture in the country leading to serious economic loss to both inland and marine aquaculture.

Shrimp farming and disease outbreaks

Shrimp cultivation in Cambodia started in the early 1990s and had increased tremendously since 1991. Black tiger shrimp (*Penaeus monodon*) and whiteleg shrimp (*Litopenaeus vannamei*) are the common species cultured in intensive and extensive systems in coastal provinces. Extensive shrimp culture system was mostly carried out by farmers in Kampot and Preah Sihanouk. Shrimp ponds are normally constructed close to the mangrove areas, some with mangroves planted inside the ponds; stocking density range from 5,000 to 20,000 postlarvae/ha. Generally, these extensive shrimp farms depend mainly on natural food propagated in the ponds for feed, and on tidal water for water change. It required low cost investment for pond construction, preparation and farm operation, without feeding, water pond aeration, and predator control for a whole cycle of shrimp cultivation or until harvesting. However, the productivity remained lower than 100 kg/ha/year. In contrast, intensive culture has stocking densities ranging from 300,000 to 500,000 postlarvae/ha. While intensive shrimp culture system was practiced predominantly by farmers in Koh Kong province located near Thailand. Intensive shrimp farming system was first introduced by Thai shrimp farmers during 1980–1990. It was an advanced system that required high capital for farm establishment, pond construction and farm operation. Moreover, high quality pelleted feed or formulated feed, water aeration and regular treated seawater exchange were required. The main species for intensive shrimp farming system was *Penaeus monodon*, at high stocking density of 300,000–500,000 larvae/ha and productions of newly established farms reached 7 to 8 metric tons (MT)/ha per crop (Lang and Sothea, 2016).

In 1999, intensive shrimp farming in Koh Kong province seriously suffered from disease outbreaks caused by White Spot Syndrome Virus (WSSV), Monodon baculovirus (MBV), and Yellowhead Disease (YHD). Black tiger shrimp was the main affected species during these outbreaks and there were many shrimp farmers who stopped their intensive shrimp farming systems and reverted to fish culture. Eventually, shrimp farming areas dropped to 850 hectares out of 1,000 hectares in the year 2000 and decreased gradually each year (Racy, 2004).

Due to *P. monodon* severely attacked by diseases leading to a substantial loss of economy to the farmers, a new alternative or complementary species with special characteristics like high disease resistance, fast growing and high tolerance to wide range of climate changes was discovered. Finally, *Penaeus monodon* was immediately replaced by *Litopenaeus vannamei* and it became a popular species at that time, but unfortunately its production gradually declined due to infection with Early Mortality Syndrome (EMS) during 2011–2013 (Lang and Sothea, 2016).

Touch (2002) reported that intensive shrimp farming in Koh Kong province increased up to 1,000 ha until the onset of white spot syndrome virus (WSSV) outbreaks and it was the most serious problem faced by farmers in Cambodia causing economic losses of approximately USD 14.5 million per year. To date,

extensive and semi-intensive shrimp farming are practiced mainly in Kampot, Kep and Preah Sihanouk and Koh Kong. Only one super intensive RAS Indoor white leg shrimp farm in Kampot started to operate in 2019.

Sea bass external parasitic diseases monitoring

Recently, Mam *et al*., (2019) studied the parasitic diseases of sea bass in the Marine Research and Development Center (MARDeC) and in the cage and pond culture systems in coastal provinces such as Koh Kong, Preah Sihanouk, and Kampot provinces. This investigation aimed to identify the types of parasitic disease which occur in MARDeC, in cage and pond culture in coastal provinces. A total of 8 different species of external parasites were found, 2 with ciliate protozoa (*Cryptocaryon irritans* and *Trichodina* sp.), 1 with crustacean sea lice (*Caligus* sp.), 1 with Crustacean copepod (*Lernaea* sp.), 2 with Capsalid monogenean (*Benedenia* sp. and *Ancyrocephalidae* sp.), 1 with Dinoflagellate (*Amyloodinium* sp.), and 1 with *Myxozoa* (*Myxozoa* sp.). This showed that 3 types of parasites such as *Ancyrocephalidae* sp., *Trichodina* sp. and *Cryptocaryon irritans* have the highest prevalence of infection followed by *Myxozoa* sp., *Caligus* sp. and *Lernaea* sp. in those coastal provinces including MARDeC, while *Amyloodinium* sp. occurred in MARDeC only.

Activities on freshwater fish disease and health monitoring program

From 2016–2018, under the European Union funded program "Promotion of Inclusive and Sustainable Growth in the Agriculture Sector: Fisheries and Livestock, DCI-ASIE/ 2012/ 023-197 Fisheries sub-sector Component, DCI-ASIE/2013/331-574 (EU-PGA-FiA)," a team of aquatic animal disease and health management from the Department of Aquaculture Development (DAD) carried out a fish health monitoring program in 10 target provinces. In each mission, a team was formed with a combination of officers from DAD and Marine Aquaculture Research and Development Center (MARDeC) and a provincial FiA officer. The current status of fish disease occurrence in the province was discussed first before conducting the fish health monitoring activities. Fish monitoring activities were divided in two main phases. Phase 1 is on-site fish sample collection phase. The activities include interviewing farm owners and inspection of fish farms (pond/cage system). Water samples were collected to test water quality parameters that mainly influenced the health of farmed fish. The team gave recommendations to the farmers for reconditioning their farm as a primary and urgent solution then finally the team collected suspected fish samples for further diagnosis. For phase 2, suspected fish samples were transported from each province to an aquatic animal health laboratory of the Marine Aquaculture Research and Development Centre (MARDeC) for further disease diagnosis. The diagnosis mainly focused on pathogenic parasites and bacteria that notoriously infected major farmed fish species. The leader of the team collected and synthesized the information from on-site monitoring/interviews and laboratory diagnosis. The leader then inform fish farmers about diagnosis and provided technical recommendations to solve the occurring problems at their fish farms. A summary of farms visited during the Diseased fish sample collection from 10 target provinces under the National Fish Disease and Health Monitoring Program funded by the European Union is presented in the table on the next page.

Province	Farm	Culture System		Sampled Species	Year
		Pond	Cage		
Kampong	17	10	7	-*Channa micropeltes*	2016
Chhnang				-*Channa striata*	
Kandal	16	16	0	-*Pangasianodon hypophthalmus*	2016
Banteay Meachey	14	14	0	-*Oreochromis niloticus*	2017-2018
Battambang	18	14	4	-*Anabas testudineus*	2017-2018
Kampong Thom	18	9	9	-*Oxyeleotris marmorata*	2017-2018
Kampong Cham	9	6	3	-*Cyprinus carpio*	2018
Pursat	14	6	8	-Hybrid catfish	2018
Prev Veng	9	6	3	(*Clarias batrachus* and *C. gariepinus*)	2018
Takeo	13	13	0	-*Pangasius larnaudii*	2018
Preah Sihanouk	11	11	0	-*Hypsibarbus pierrei*	2018
Total	139	105	34		

Sources: (Khan *et al*., 2019)

During the monitoring program, Marine Aquaculture Research and Development Center (MARDeC) was the only main laboratory for aquatic animal health diagnosis. Some diseases such as Bacillary necrosis of pangasius (BNP), Red spot or Motile aeromonas septicemia, are of concern in farmed aquatic animals of Cambodia at that time. Several pathogens have been identified from collected farmed fish such as Fish parasites: *Trichodina* sp., *Ichthyophthirius multifiliis*, *Epistylis*, *Apiosoma*, *Dactylogyrus* sp., *Gyrodactylus* sp., *Lernaea* sp., *Argulus* sp. and Acanthocephalan. Fish pathogenic fungi: *Aphanomyces invadans* and *Saprolegnia* sp. Fish pathogenic bacteria: *Aeromonas* spp. and *Edwardsiella ictaluri*. Marine Aquaculture Research and Development Center (MARDeC) was the only main laboratory for aquatic animal health diagnosis. To date, diagnostic laboratories can perform level I and II but not for all species of aquatic animals and diseases, whereas level III is not yet effectively performed due to the lack of facilities, skills and knowledge.

Conclusion and way forward

Intensive shrimp farming systems along the Cambodian coastal line have been severely affected by diseases due to limitation of aquaculture skills, GAP and biosecurity practices. To date, extensive and semi-intensive shrimp farming have started mainly in Kampot, Kep and Preah Sihanouk and Koh Kong. Only one super intensive RAS Indoor white leg shrimp farm in Kampot operated in 2019. According to the recent research on fish disease and health management program, commercial freshwater and marine fish species in floating net cage

and pond systems suffered from some external parasitic and bacterial diseases. Concerning the diagnostic capability, the officers can perform level I and II but not all aquatic animals and diseases, also level III it is not yet effectively performed.

Disease is considered as a major problem in developing aquaculture in Cambodia because it has caused serious losses in the livelihood of farmers. Thus, to minimize the spread of disease, outbreaks and promote the expansion of aquaculture production in Cambodia. The tasks for sustainable aquaculture should be performed including:

- Improvement, amendment, and enforcement of the regulations, laws, SOPs and other relevant documents for responsible management to establish the aquatic emergency preparedness and response systems for effective management of transboundary disease outbreaks in Cambodia.
- Capacity building of aquatic animal health management officers on technique and skills for monitoring and disease surveillance.
- Development of national guidelines on Good Aquaculture Practice (GAqP), Biosecurity practice and management of chemical and organic residues, and invasive species in Cambodia.
- Establishment of laboratory for aquatic animal health monitoring, diagnosis and analysis, extension services and seeking for continuous funding for research.
- Strengthening collaborations among central officers, provincial officers, researchers, farmers' networks, ASEAN member states and other NGOs to have good technical expertise particularly aquatic animal health management.

References

Fisheries Administration. 2017. National Strategic Plan for Aquaculture Development in Cambodia (2016 to 2030). Phnom Penh, Cambodia: Ministry of Agriculture, Forestry and Fisheries.

Fisheries Administration. 2015. The Strategic Planning Framework for Fisheries: 2015-2024 Cambodia, Phnom Penh, Cambodia: Ministry of Agriculture, Forestry and Fisheries

Hav, V., & Leap, H. 2005. Status of shrimp farming in Cambodia, pp. 38-41. In Regional Technical Consultation on the aquaculture of *P. vannamei* and other exotic shrimps in Southeast Asia, Manila, Philippines. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines.

Hortle, KG. 2007. Consumption and the Yield of Fish and Other Aquatic Animals from the Lower Mekong Basin. MRC Technical Paper No. 16. Vientiane, Lao People's Democratic Republic: Mekong River Commission.

Joffre, OM., Pant, J., Somony, T., Chantrea, B. and Viseth, H. 2019. Transforming aquaculture in Cambodia through introduction of improved tilapia. Penang, Malaysia: World Fish. Program Brief: 2019-03.

Khan, C. D., Chhorn, S., & Thay, S. 2019. Current status, issues, and gaps on aquatic emergency preparedness and response systems practiced by Cambodia. In E. A. Tendencia, L. D. de la Peña, & J. M. V. de la Cruz (Eds.), Aquatic Emergency Preparedness and Response Systems for Effective Management of Transboundary Disease Outbreaks in Southeast Asia: Proceedings of ASEAN Regional Technical Consultation, 20-22 August 2018, Centara Grand Central Ladprao, Bangkok, Thailand (pp. 7-11). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Lang, O. 2015. Current status of sustainable aquaculture in Cambodia. In M. R. R. Romana-Eguia, F. D. Parado-Estepa, N. D. Salayo, & M. J. H. Lebata-Ramos (Eds.), Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA) (pp. 27-40). Tigbauan, Iloilo, Philippines: Aquaculture Dept., Southeast Asian Fisheries Development Center.

Lang, O. & Sothea, M. 2016. Current status of shrimp farming and diseases in Cambodia. In R. V. Pakingking Jr., E. G. T. de Jesus-Ayson, & B. O. Acosta (Eds.), Addressing Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia: Proceedings of the ASEAN 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 (pp. 33-36). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Mam, S, Ao, V. & Prom, C. 2019. Parasitic disease of seabass (*Lates calcarifer*) in coastal aquaculture of Cambodia. In: Cambodia Fisheries Magazine, Vol. 26 (June-December) 2019, Year 16, 1st-2nd Semester, pp: 61-67.

Racy, B. 2004. Current status of transboundary fish diseases in Cambodia: Occurrence, surveillance, research and training. In C. R. Lavilla-Pitogo & K. Nagasawa (Eds.), Transboundary Fish Diseases in Southeast Asia: Occurence, Surveillance, Research and Training. Proceedings of the Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurence, Surveillance, Research and Training, Manila, Philippines, 23-24 June 2004 (pp. 85-89). Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Rainboth, W.J. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome.

Sorphea, S., Kiessling A., Barnes A. C., Da, C. T., Lindberg, J. E. and Lundh, T. 2018: A field survey of small-scale cage and pond farming of Asian Seabass (*Lates calcarifer*) in Cambodia. Livestock Research for Rural Development. 30.

Touch, S. T. 2002. The inland and marine fisheries trade of Cambodia.

Try, I. 2003. Fish stocks and habitats of regional, global and transboundary significance in the South China Sea (Cambodia). In: Reversing environmental degradation trends in the South China Sea and Gulf of Thailand. UNEP and Global Environment Facility.

Status of Aquatic Animal Health in Indonesia

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Abstract

Fish disease is one of the main obstacles in the success of aquaculture production because of the loss caused by it. The outbreak of diseases has resulted to a substantial economic loss which was reported to have reached almost USD 400 million. To minimize the impact of losses caused by fish diseases, the Indonesian government through the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries has a fish disease monitoring and surveillance program. The program aims to monitor the occurrence of fish diseases in Indonesia, especially in the fish and shrimp farming centers and to educate on how to control them. In 2018, the monitoring and surveillance program have 34 provinces with 100 districts/cities location targets targeting fish and shrimp diseases. Based on the results of the monitoring and surveillance activities in 2018, the fish and shrimp are affected by the following diseases: White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Haemotopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV), Iridovirus, *Aeromonas hydrophila*, *Streptococcus iniae*, *Streptococcus agalactiae*, *Edwardsiella ictaluri* and Ichthyophthiriasis. The program to control fish diseases in order to minimize the losses has also been carried out by the government including trainings on the application of biosecurity, the use of vaccines, probiotics, immunostimulants and herbal medicines.

Introduction

Indonesia is an archipelago with 17,504 islands and 99,093 km coastline as a large asset to develop aquaculture. Aquaculture in the fisheries subsector has been growing rapidly lately, due to efforts to increase production which has led to intensive aquaculture. In intensive aquaculture, success in production is largely determined by several factors, including seeds, water quality, and aquaculture management. However, obstacle become one of the determinants of failure in aquaculture. The obstacles that can be found in the field such as availability of inadequate seedlings and fish diseases which can inhibit the increase of national fisheries production and detrimental fish and shrimp farmers in Indonesia.

Disease is one of the main obstacles in the success of aquaculture production because of the loss caused by it. The emergence of disease is a dynamic process and interaction between the host, pathogen, and the environment. In nature, the three factors are balanced, so there is no disease. Diseases that are often found in fish and shrimp farming in Indonesia are infectious and non-infectious diseases. Infectious disease is a disease caused by

microorganisms, such as bacteria, fungi, parasites, and viruses. On the other hand, non-infectious diseases that can be found in fish farming, are those related to the environment or nutrition. Infectious diseases can cause death and the rates are reaching 90–100 %, causing huge losses to fish and shrimp farmers.

The outbreak of diseases has resulted to a substantial economic loss which was reported to have reached almost USD 400 million (Lusiastuti *et al*., 2020). To minimize the impact of losses caused by fish diseases, the Indonesian government through the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries has a fish disease monitoring and surveillance program. The program aims to monitor the occurrence of fish diseases in Indonesia, especially in fish and shrimp farming centers and to educate on how to control them. In 2018, the monitoring and surveillance program targeting fish and shrimp diseases included 34 provinces and 100 districts/cities. Based on the results of monitoring and surveillance activities in 2018, shrimp are affected by the following diseases: White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Haemotopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV); fish with Iridovirus, *Aeromonas hydrophila*, *Streptococcus iniae*, *Streptococcus agalactiae*, *Edwardsiella ictaluri* and Ichthyophthiriasis.

In addition, a program to control fish diseases in order to minimize the losses has also been carried out by the government. Among these are trainings on the application of biosecurity measures, the use of vaccines, probiotics, immunostimulants and herbal medicines. Biosecurity is one of the key factors in preventing the entry and spread of fish and shrimp diseases. The use of vaccines, probiotics and immunostimulants are useful to improve the immune system of fish and shrimp making them more resistant to diseases. Natural herbal medicines have been used to replace antibiotics as antibacterial. The following provides details on the current condition of fish and shrimp diseases in Indonesia.

Shrimp diseases

One of the factors causing failure in shrimp farming in ponds is diseases. The most dangerous and detrimental disease for farmers are viral infections (WSSV, TSV, YHD, IMNV, and IHHNV). Based on the results of monitoring and surveillance activities in 2018, diseases affecting shrimps are: White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Haemotopoietic Necrosis Virus (IHHNV), and Infectious Myonecrosis Virus (IMNV).

White spot syndrome virus (WSSV)

In 2018, WSSV disease was found in several shrimp pond centers, the areas affected by this disease includes: Aceh, West Java, Central Java, East Java, Yogyakarta, Bali, South Sulawesi and West Nusa Tenggara. The average mortality rate reached 60–100 %, causing high economic losses. Economic losses in 2016 due to WSSV outbreak were approximately 300 million USD (Shinn *et al*., 2018). White Spot Syndrome (WSS) is a viral disease in penaeid shrimp farming, especially tiger shrimp (*Penaeus monodon*), kuruma shrimp (*P. japonicus*), *P. chinensis*, *P. indicus*, and white leg shrimp (*Litopenaeus vannamei*). This disease, caused by a Baculovirus, is very virulent with mortality reaching 100% in just few days after infection. Some fresh and seawater crustaceans, as well as shrimp that survive after being infected with this disease, usually have the potential to be carriers of white spot disease (OIE, 2019). The attack of white spot disease in Indonesia was first reported in areas of tiger shrimp farms in Tangerang, Serang, and Karawang in mid 1994 (Mahardika *et al*., 2004). At present, WSSV was believed to have spread to various shrimp ponds throughout Indonesia.

Transmission usually occurs horizontally through cannibalism in shrimp. Water and wild crustaceans, such as crabs, can also be a factor in the spread of disease. The WSS disease agent can survive in the environment for 3–4 days (OIE, 2019). In acute cases, feed consumption was significantly decreased. Infected shrimps are weak, swimming near the surface and undirected. In addition, white spots are usually found on the carapace and rostrum of shrimp when entering the sub-acute and chronic phases. Dying shrimp had brownish red colour (Bondad-Reantaso *et al*., 2001).

Polymerase Chain Reaction (PCR) was used to detect White Spot Syndrome (WSS), in addition to observing the clinical signs.

Infectious hypodermal and haemotopoietic necrosis virus (IHHNV)

In 2018, shrimp infected with IHHNV was found in West Java, Banten and South Sulawesi with an average mortality rate of 30-60%. IHHNV is a disease in penaeid shrimp caused by Parvovirus. Transmission of IHHNV can occur both vertically, through the female reproductive organs, or horizontally, through cannibalism or cohabitation in water (OIE, 2019). IHHNV was first discovered infecting *P. vannamei* postlarvae in the hatchery and broodstock on Situbondo, East Java in 2003. IHHNV infection in shrimp can cause retardation of shrimp growth. Observed clinical signs are decrease in feed consumption, slow growth, swim on the surface, and abnormal swimming behavior. Further observation includes: white patches found between the exoskeleton and carapace segments. In addition, white leg shrimp are non-uniform in size, have bent rostrum, and rough cuticles (Bondad-Reantaso *et al*., 2001).

Diagnosis of IHHNV can be done by observation of clinical signs and molecular laboratory testing via PCR.

Infectious myonecrosis virus (IMNV)

In 2018, IMNV disease was found in several shrimp pond centers. Areas affected by this disease includes Central Java, East Java, Banten, South Sulawesi, Bali and West Nusa Tenggara with an average mortality rate of 30–60 %. Data on economic losses in 2016 due to IMNV disease amounted to USD 95.6 million (Shinn *et al*., 2018). This IMNV disease is caused by a Toti-like virus from the Totiviridae family, with both vertical and horizontal transmission. The pattern of disease attacks can either be acute or chronic. The acute attack marked by clinical signs and mortality reaching 60–85 %, while chronic marked by low mortality but persistent (OIE, 2019). IMNV was first discovered in the white leg shrimp ponds at Situbondo, East Java, Indonesia in 2006 (Tang *et al*., 2019). Specific clinical signs of Infectious Myonecrosis Virus (IMNV) disease are white (necrotic) areas in the muscles that resemble strokes, especially in the last 1/3 of shrimp body parts. In addition, muscle necrosis is also sometimes observed as a red discoloration (looks like boiled or cooked) of the tail uscle with a firm boundary (OIE, 2019).

Observation of specific clinical signs and testing using the PCR method are used to diagnose Infectious Myonecrosis Virus (IMNV).

Fish diseases

Based on the results of monitoring and surveillance activities in 2018, diseases affecting the cultured fishes are: Iridovirus, *Aeromonas hydrophila*, *Streptococcus iniae*, *Streptococcus agalactiae*, *Edwardsiella ictaluri* and Ichthyophthiriasis.

Iridovirus

In 2018, spread of iridovirus are in the islands of Riau and West Nusa Tenggara, infecting large grouper and snapper fish with a mortality rate of 30 %. The first case of Iridovirus infection in grouper was reported in North Sumatra by Rukyani *et al*. (1993) In 2001, the viral infection spread to Bali and infect grouper seeds (Mahardika *et al*., 2001). Data on economic losses in 2014 due to the attack of iridovirus is USD 12.5 million. Iridovirus in brackish or sea water fish was caused by a virus from the genus Ranavirus which has a diameter approximately 160–200 nm. Viral replication occurs in the cytoplasm of infected cells and can grow well *in vitro* in tissue culture derived from groupers.

Fish that are very susceptible to iridovirus which includes snapper and grouper. Transmission of this virus can occur through direct contact or contaminated water. The target organs of this disease are the kidneys and lymph which can lead to systematic infection that can can infect other organs, such as the liver, heart, thymus, stomach, and intestines. The mortality rates of this disease ranges between 0 % and 100 % depending on factors such as host fish species, fish size, fish age, water temperature, and other culture conditions (OIE, 2019).

Common clinical signs found in fish infected with iridovirus are decreased appetite, weak swimming movements and coordination, and fish lying on one side of the body found at the bottom of the pond. The dark body color of the fish, anemia (pale gill color), and swelling of the spleen organs are the specific clinical signs of this disease (OIE, 2019).

Recognition of clinical signs with the help of molecular testing, such as PCR, is one of the most effective ways in diagnosing this disease.

Aeromonas hydrophila

Motile Aeromonas Septicemia (MAS) is a disease caused by *Aeromonas hydrophila* bacteria which has spread in several regions of Indonesia including West Java, Central Java, Banten, Jambi and South Kalimantan. This disease has been reported to have infected dumbo catfish seeds with a mortality rate between 80–100 % (Kusdarwati *et al*., 2018). MAS was first plagued Indonesia in 1980 in carp (Djajadiredja *et al*., 1983). *Aeromonas hydrophila* is a gram-negative bacteria that are native inhabitants of aquatic environments. They are often found in waters with high levels of organic matter and sewage (Scullion, 2008).

Aeromonas hydrophila has been linked to several diseases in fish, including tail rot, fin rot, and haemorrhagic septicemia. Haemorrhagic septicemia is characterized by a small surface wound, often leading to flaking of the scales, bleeding in the gills and rectum, ulcers, exophthalmia (swollen eyes), and swelling of the stomach. On the inside, it is possible to have ascitic fluid in the peritoneal cavity, lack of red blood cells, and swelling of the kidneys and liver (Stratev and Odeyemi, 2016).

The detection method used was isolation and identification of bacteria through biochemical testing or molecular methods through PCR technique.

Streptococcosis

Streptococcosis disease in 2018 spread in the South Kalimantan region that infect tilapia aquaculture commodities with an average mortality rate of 30–60 %. Based on 2014 reports, economic losses due to Streptococcosis disease reached USD 1.2 million. Supriyadi *et al*., (2002) reported that Streptococciasis disease first became an epidemic in tilapia cultivation centers

throughout Java in 2002. Streptococcosis is caused by *Streptococcus agalactiae* and *S. iniae. S. iniae* infection often occurs in sea water aquaculture; whereas *S. agalactiae* was more commonly found in freshwater aquaculture. This bacterium is one type of bacteria that causes quite serious diseases in several types of fish, including tilapia. Fish infected with this bacterium generally die (survival of less than 50 %) within 3–7 days (Yanong and Francis-Floyd, 2002). Clinical signs of fish with streptococcus infection includes hemorrhage, exopthalmia, melanosis, ulcers, or sores on the surface of the body, loss of orientation, damage to the structure of the spine, anorexia, and damage to the brain (Mishra *et al*., 2018).

The detection method used was isolation and identification of bacteria through biochemical testing or using molecular methods through the PCR technique.

Edwardsiella ictaluri

Edwardsiella ictaluri bacteria in 2018 was known to infect catfish farming centers in the South Kalimantan region with a mortality rate reaching 30–60 %. *E. ictaluri* is a bacterium that causes systemic bacterial disease called enteric septicemia of catfish (ESC). This bacterium was initially known to infect cannel catfish, but was later known to infect other types of fish such as other species of catfish and eel. Based on experiments, some types of fish such as trout, tilapia, salmon and ornamental fish can also be infected with this type of bacteria. *E. ictaluri* was first discovered in Indonesia in 2002 (Sakai *et al*., 2009), infecting catfish in the Jambi region and also caused significant economic losses. In South Kalimantan and Central Kalimantan was also reported that in 2012 it had infected catfish farming and caused more than 50 % mortality. Clinical signs of fish infected with *E. ictaluri* includes: appearance of red spots on the body of the fish, swelling of the abdomen and fish swimming without direction/ whirling (Hawke *et al*., 2013).

The detection method used was isolation and identification of bacteria through biochemical testing or using molecular methods through PCR technique.

Ichthyophthiriasis

In 2018, Ichthyophthiriasis spread almost in all regions of Indonesia. The affected areas include Jambi, Bengkulu, Jakarta, West Java, Central Java, East Java, South Kalimantan and Yogyakarta. This disease attacks the centers of freshwater aquaculture with a mortality rate of 30–60 %. This disease was caused by the parasitic *Ichtyophtyrius multifilis. I. multifilis* belongs to the parasitic ecto group, a shaggy-haired protozoan, an obligate parasite in freshwater that must find a new host within 48 hours (at 25–27 °C). The parasite has hairy structures (cilia) and horseshoe-like core. This parasite measures 30 µm × 50 µm covered with cilia. This parasite infests the skin, fins, and gills. Clinically, infected fish become hyperactive and irritably swim while rubbing their bodies against rocks or aquarium walls, epidermal hyperplasia, and has white spots on the skin or gills (Klinger and Francis-Floyd, 2009).

An infected fish lose appetite and becomes weak or has a decreased activity. In severe infections, especially if the attack is on the gills, the gills become pale and swollen. This interferes with oxygen absorption, resulting in respiratory distress and eventually to death. This parasite is known as white spot disease or ich and is very common in domesticated fish in aquariums or in hatcheries. Ichthyophthiriasis or ich is very contagious and spreads rapidly (Francis-Floyd and Reed, 2009).

Figure 1. Fish and shrimp deaths due to disease attacks

Handling of fish and shrimp disease in Indonesia

In terms of efforts to control fish diseases, the government continues to conduct trainings related to the application of biosecurity measures in aquaculture. The concept of biosecurity is the most appropriate step to reduce the entry of pathogens in aquaculture environment and prevent its spread to other places. The principle of applying biosecurity includes knowledge of the disease, list of diseases, availability of tools/methods for detection of pathogens, control of the pathogen, management of the environment, application of Best Management Practices (BMP), disease eradication programs, and disinfection of pathogens. The application of biosecurity measures thoroughly and strictly in the aquculture environment is one of the effective efforts that can be done during the production process. The Indonesian government also promotes the use of vaccines, probiotics, immunostimulants and natural medicines in the context of preventing and treating fish diseases. In addition, zoning of free and infected areas is based on monitoring and surveillance of fish diseases carried out by the government and limiting the entry and exit of shrimp to and from the free zone (quarantine).

In an effort to detect the presence of fish diseases, the Indonesian government under the Ministry of Marine Affairs and Fisheries has 91 fish disease testing laboratories spread throughout Indonesia. Efforts to control fish diseases in the aquaculture area are inseparable from the important role of the laboratory which can have significant benefits and have an extraordinary impact on the community through laboratory testing services at the aquaculture center. The fish disease testing laboratory supports the success of the program to increase aquaculture production through water quality monitoring and surveillance activities, inspection of fish diseases, as well as providing recommendations for the prevention of fish diseases and sustainability of the environment. Through these activities, it is expected to improve the ability of the early warning system and early response, so that the possibility of outbreaks of fish diseases and pollution of the aquaculture environment can be immediately handle.

Emergency response of exotic fish disease in Indonesia

The problem of fish and shrimp diseases in aquaculture businesses is increasing which is caused by several problems. This includes an increase in the area of aquaculture, a large number of live fish trade, intensive aquaculture, less intensive monitoring and surveillance efforts, and the entry of new fish commodities that are not accompanied by Import Risk Analysis (IRA) studies. In addition, quarantine acts are not supported by adequate equipment and limited information of the farmers in the effort to control the disease. Moreover, the problem of pollution in the aquaculture environment contributes to the susceptibility of the cultured organism. Alongside the widespread of diseases in the area of aquaculture, it is necessary to immediately implement policies and strategies for fish health management so that fish are kept protected from diseases.

Indonesia continues the efforts to prevent the entry of diseases in shrimp that have potential risk and can reduce the quality and quantity of shrimp aquaculture production in Indonesia. One of the concerns is acute hepatopancreatic necrosis disease (AHPND). AHPND has now spread to several countries such as China, Thailand, Vietnam, Malaysia, Mexico and the Philippines. So, the Indonesian government continues to make efforts to disseminate to all shrimp farming communities related to the prevention of AHPND disease. In addition, the Indonesian government made regulations regarding the prevention of new diseases that had not yet entered Indonesian territory and had become epidemic in several countries. This enables the government to form a task force for AHPND disease prevention consisting of government elements, stakeholders, and academic experts.

Figure 2. Training on the application of biosecurity measures and the use of vaccines, probiotics, and immunostimulants

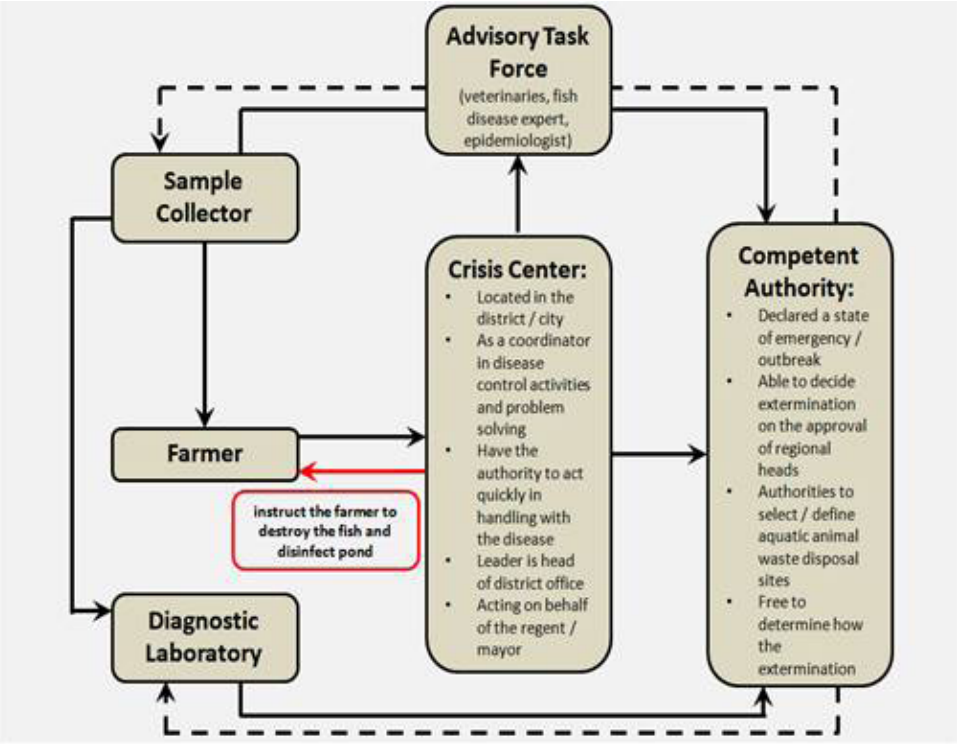

Figure 3. Task Force Institution in Emergency Response of Fish Disease

References

Beers, P; V. Findlay; R. Perera. 2002. *Biosecurity. A new word for an old concept.In* Handbook and Abstracts of the 5th Symposium on Diseases in Asian Aquaculture. Gold Coast, Queensland.

Bondad-Reantaso, M.G., McGladdery, S.E., East, I., and Subasinghe., R.P. (eds). 2001. Asia diagnositic guide to aquatic animal disease. FAO Fisheries Technical Paper No. 42. Supplement 2. Roma. FAO.

Deborah B, Pouder; Eric W. Curtis, dan Roy P.E. Yanong., 2005. *Common Freshwater Fish Parasites Pictorial Guide: Monogneans.* FA-111. University of Florida. IFAS Extension.

Djajadiredja R., Panjaitan T.H., Rukyani A., Sarono A., Satyani D. & Supriyadi H. 1983. In: Fish quarantine and fish disease in Southeast Asia. International Development Research Center, Ottawa, Canada. Country reports: Indonesia,p. 19-30

Dvorak, Glenda. 2009. *Biosecurity for Aquaculture Facilities in the North Central*Region. North Central Regional Aquaculture Center In Cooperation withUSDA. Fact Sheet Series 115.

Ellis, A.E. (Ed). 1988. *Fish Vaccination*. Academic Press, San Diego, New York.

Fuller, R. 1989. A. review, Probiotics in an and animals. *Journal of Microbiology*,66: 365-378.

Hawke JP, Kent M, Rogge M, *et al*. 2013. Edwardsiellosis caused by Edwardsiella ictaluri in laboratory populations of Zebrafish Danio rerio. J Aquat Anim Health. 25(3):171-183. doi:10.1080/08997659.2013.782226

Klinger, RE and Francis-Floyd R. 2009. *Introduction to Freshwater Fish Parasites.* CIR-716. University of Florida. IFAS Extension.

Kusdarwati, Rahayu & Rozi, Rozi & Dinda, N & Nurjanah, I. 2018. Antimicrobial resistance prevalence of Aeromonas hydrophila isolates from motile Aeromonas septicemia disease. IOP Conference Series: Earth and Environmental Science. 137. 012076. 10.1088/1755-1315/137/1/012076.

Francis-Floyd R and Reed P. 2009. *Ichthyophthirius multifillis* (White Spot) in Fish. CIR-920. University of Florida. IFAS Extension.

Lusiastuti A, Taukhid, Maskur, Murwantoko, Prayitno S, Sugiani D, Caruso D. 2020. Building and improving the capacity of fish and environmental health management strategy in Indonesia. IOP Conference Series: Earth and Environmental Science. 521. 012016. 10.1088/1755-1315/521/1/012016

Mahardika K, Zafran, Koesharyani I. 2004. Deteksi *White Spot Syndrome Virus* (WSSV) pada udang windu menggunakan metode PCR. Jurnal Penelitian Perikanan Indonesia 10 : 55-60

Mahardika K, Koesharyani I, Sugama K, Priyono A, Yuasa K. 2001. Histopathological study of iridovirus infection in Epinephelus coioides and Epinephelus bleekeri. Proceedings of Mariculture Technology and Sea Farming Development. Jakarta, Indonesia. Japan International Cooperational Agency, Jakarta, p 334–341 (in Indonesian with English abstract)

Mishra A, Nam GH, Gim JA, Lee HE, Jo A, & Kim HS. 2018. Current Challenges of Streptococcus Infection and Effective Molecular, Cellular, and Environmental Control Methods in Aquaculture. Molecules and cells, 41(6), 495–505. https://doi.org/10.14348/molcells.2018.2154

Noga, Edward J. 2000. Fish Disease : Diagnosis and Treatment. Iowa State University Press.

Rukyani A, Taufik P, Yuliansyah A. 1993. Laporan Survei kasus kematian ikan kerapu di daerah Sumatera Utara. 12p.

Sakai T, Yuasa K, Ozaki A, Sano M, Okuda R, Nakai T, Lida T. 2009. Genotyping of *Edwardsiella ictaluri* isolates in Japan using amplified-fragment length polymorphism analysis. Letter in Applied Microbiology 49:443-1449.

Scullion FT. 2008. Selected Fish Diseases in Wild Populations. Zoo and Wild Animal Medicine (Sixth Edition) 112-120. doi:https://doi.org/10.1016/B978-141604047-7.50017-8

Shinn A, Pratoomyot J, Griffiths D, Trong T, Vu N, Jiravanichpaisal P, & Briggs M. 2018. Asian shrimp production and the economic costs of disease. Retrieved from http://www.asianfisheriessociety.org/publication/abstract.php?id=1223

Stratev D, & Odeyemi OA. 2016. An overview of motile *Aeromonas septicaemia* management. Aquaculture International 2017 Vol.25 No.3 pp.1095-1105 ref.78. doi 10.1007/s10499-016-0100-3

Sunarto A, Widodo, Taukhid, Koesharyani I, Supriyadi H, Gardenia L., ... Rukmono D. 2004. Current status of transboundary fish diseases in Indonesia: Occurrence, surveillance, research and training. In C. R. Lavilla-Pitogo & K. Nagasawa (Eds.), Transboundary Fish Diseases in Southeast Asia: Occurence, Surveillance, Research and Training. Proceedings of the Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurence, Surveillance, Research and Training, Manila, Philippines, 23-24 June 2004 (pp. 91-121). Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Supriyadi, H., Effendie, J., dan Bastiawan, D. 2002. Penyebaran Penyakit Streptococcosis pada Beberapa Pusat Budidaya Ikan Air Tawar. [Laporan teknis]. Balai Riset Perikanan Budidaya Air Tawar. Bogor. 1-6.

Tang K.F.J., Bondad-Reantaso M.G. & Arthur J.R. 2019. Shrimp infectious myonecrosis strategy manual. FAO Fisheries and Aquaculture Circular No. 1187. Rome, FAO.

Yanong, R. & Francis-Floyd, R. 2002. Streptococcal Infections of Fish 1.

Zafran, Des Roza, Koesharyani, I., Johnny, F and Yuasa, K. 1998. Manual for Fish Diseases Diagnosis. Marine fish Crustacean Diseases in Indonesia. Gondol Research Station for Coastal Fisheries and JICA.

Report on Aquatic Animal Health in Lao PDR

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Abstract

Fish production is very important to Lao PDR. It is an important source of protein to its citizens. Lao PDR is fortunate to have numerous water resources. The Mekong River flows through Lao PDR for a length of 1,865 km. Additionally, the country has other rivers, streams, reservoirs which are also used for irrigation and source of hydroelectric power. Swamps, lakes and rice fields during the wet season provide for capture and cultured fish products. In 2018, these water resources produced a total of 179,100 tons of fish; of which 62,700 came from capture fisheries and 116,400 from fish culture.

In Lao PDR, as in many countries throughout the world, inland fisheries and aquaculture activities are administered by the Ministry of Agriculture. The Ministry is also responsible for forestry. It is called the Ministry of Agriculture and Forestry and is referred to as MAF. Within the MAF is the Division of Livestock and Fisheries (DLF). The National Fisheries Development Centre (NFDC) is working diligently to prevent outbreaks of aquatic animal diseases but much more must be done. Adequate manpower to address fisheries disease control on fish farms and adequate manpower for drug and chemical testing and inspection are lacking. In addition, communication between central and local governments as well as between local and central governments regarding disease diagnosis and disease control needs to be enhanced. Fish farmers must be trained to recognize and control diseases.

Country Report Aquatic Animal Health in Malaysia

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Abstract

The fisheries sector of Malaysia plays a significant role in economic development. It provides employment, foreign exchange and protein supply for the country. In 2017, aquaculture production in Malaysia was 427,022 tonnes, a 4.8 % increase compared to 2016. The increase was driven by population growth, rising demand for seafood and a levelling of production from capture fisheries. However, the rapid growth of aquaculture has been source of anthropogenic change on a massive scale. Aquatic animals cultured in high density are exposed to environment stress leading to diseases. Among major diseases occur in Malaysia are TILV and Streptococcosis in Tilapia, Vibriosis in grouper, and APHND and EHP in shrimp. Losses due to these diseases were reported as USD 0.1 billion for APHND in 2011, MYR 1 million due to Streptococcosis in 2002 and USD 7.4 million in Vibriosis outbreak in 1990. Currently the use of chemicals to overcome these diseases by farmers has led to increase concerns on food safety of food fish. Thus, Malaysia has implemented strict biosecurity measures in fisheries practices to secure not only fish health but also food safety for the consumers. This paper aimed to discuss the status of fish diseases and national diseases response and surveillance in Malaysia.

Aquatic Animal Health in Myanmar

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Abstract

In 2010, several viruses infected *Penaeus monodon* in the ponds of Myanmar. This includes the White Spot Syndrome Virus (WSSV) which causes the White Spot Disease (WSD). In addition, Taura Syndrome Virus (TSV) and Infectious Hypodermal and Haematopoietic Virus (IHHNV) were detected in *P. monodon* samples from Ayeyarwaddy Region (western part of Myanmar). In 2014, the Yellowhead Virus (YHV) was also detected in shrimp samples for export. The occurrence of these shrimp diseases has resulted to a devastation of the shrimp industry in Myanmar. Because of this, most of the shrimp farmers have shifted to extensive or traditional shrimp farming. The Aquatic Animal Health and Disease Control Section (AAHDCS) of the Department of Fisheries (DoF) is responsible for formulating action plans to control and prevent aquatic animal diseases. Thus, in order to be updated with the latest techniques on disease detection and management of emerging diseases, the AAHDCS should improve the capacity of the departmental personnel, upgrade the laboratory equipment, and improve the facilities.

Introduction

Myanmar is a country that is rich in marine and inland fishery resources with a total of 486,000 square kilometers of marine fishery areas and a coastline of 2,832 kilometers. The country also has several inland water bodies such as natural lakes, reservoirs, rivers, and ponds which cover an area of about 8.1 million hectares. Thousands of families in Myanmar rely on inland fisheries as their livelihood. Moreover, inland fisheries also contribute to the national revenue which led to an effort by the government of Myanmar to increase fish production through culture-based capture fisheries.

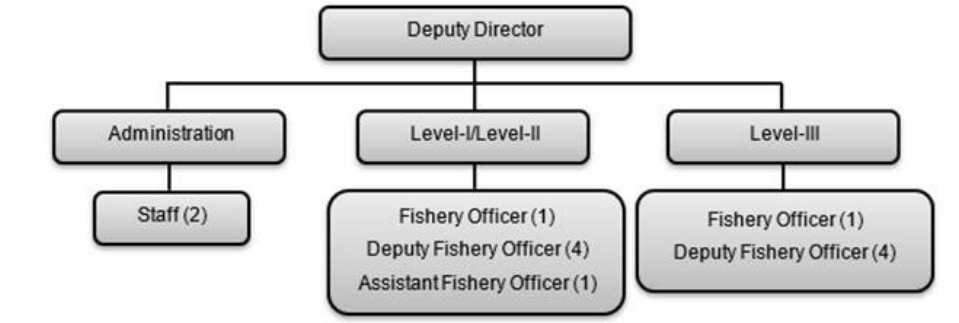
Aquaculture is one of the main contributors to the national economy of Myanmar. The land area for fish and shrimp ponds are approximately 91,653 ha and 92,681 ha, respectively (Wah & Than, 2016). The country is known for its production of freshwater fishes, including the culture of rohu (*Labeo rohita*). These freshwater species do not only provide supply for domestic consumption, but also

for neighboring countries. However, the people of Myanmar prefer freshwater fish over marine fish, thus the Government of Myanmar laid out a policy to target marine fish for the export market.

In the 1970s, shrimp culture in Myanmar started by using a trap and hold method. Postlarvae (PL) of *Penaeus monodon* were trapped into ponds during high tide. However, there were no available information on pond preparation, eradication of predators, water fertilization, and feeding. As the ponds were usually as large as 50 to 100 ha, shrimp production provided more than enough income for the shrimp farmers (Thame & Maye, 2005).

In 2010, the Department of Fisheries, Ministry of Agriculture Livestock and Irrigation (MOALI) implemented a 3-year project which aims to develop three types of shrimp farming: extensive, extensive plus, and semi-intensive (Wah & Than, 2016). This was followed by another 3-year project which focuses on the development of an intensive culture system (Saw, 2004). The total area for semi-intensive shrimp ponds is 24,536.29 ha, 61,059.63 for extensive plus shrimp ponds, and 169,818.54 ha for extensive or traditional shrimp ponds with a total land area of 235,474.46 ha. The total production of freshwater prawn and marine shrimp in 2017 was 35,694.20 metric tons. Recently, the Department of Fisheries encouraged the practice of fish and shrimp culture in every states and regions for self-sufficiency of local consumption and increasing for export market (DOF, 2017). However, in recent years, many hatcheries including private and public are facing difficulties on availability of sufficient amount of shrimp broodstocks when required. Therefore, local shrimp hatcheries could not produce enough shrimp seeds for local demand and shrimp post larvae had to be imported from neighboring countries such as Thailand and Bangladesh. Import numbers of shrimp larvae from Bangladesh is not yet available. In 2017–2018, tiger shrimp, freshwater prawn and white shrimp larvae were imported (57.99 million) from Thailand. *Penaeus vannamei* has many advantageous factors for culture but it may also cause the negative impact to other shrimp aquaculture industry. DOF has been aware that *P. vannamei* may be a carrier and may result in the outbreak of TSV. After a regional workshop in 2005 in Manila that assessed the culture of *P. vannamei*, ASEAN countries agreed to culture with proper documentation. At present 3–4 private farms are culturing *P. vannamei* at experimental scale. Only PCR negative SPF *P. vannamei* seeds are permitted to be imported for culture in domestic water. In 2017–2018, a total of 61.58 million *P. vannamei* larva were imported (Regional Technical Consultation, 2005). Recently, the most prominent development of white shrimp culture is in Tanintharyi Region at Pyay Pho Tun Co. Ltd. The company initiated the farming of white shrimp in 2016 and continuously invested in its production. In 2017, the company produced 1334.936 MT of white shrimp and 1,006,194 MT in 2018.

The Department of Fisheries (DoF) is responsible for the management and sustainability of the fisheries development in Myanmar. The major goal of the department is to enhance food security by increasing fish production not only for domestic consumption but for export as well. The DoF also focuses on forming strategies to promote proper aquatic animal health management so that the occurrence of disease in hatcheries and grow-out facilities can be controlled and prevented (Wah & Than, 2016).



Level	Site	Activity
I	Field	Observation of animal and the environment, Clinical Examination and Extension Service
II	Lab	Diagnosis of Parasitology, Bacteriology, Mycology, Histopathology, and Extension Service
III	Lab	Diagnosis of PCR and Extension Service

Technician	= 12
Staffs	= 2
Total	= 14

Figure 1. Organizational chart of the Aquatic Animal Health and Disease Control Section of the Department of Fisheries in 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 must be undertaken.

Shrimp disease in Myanmar

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 White Spot Disease (WSD), Infectious Hypodermal and Haematopoietic Necrosis (IHHN), Taura Syndrome (TS), and Yellowhead Disease (YHD). In addition, the AAHDCS laboratory plans to establish the diagnostic methods for AHPND following the suggested methods in the OIE Manual.

Fish disease in Myanmar

Viral Disease

Epizootic Ulcerative Syndrome (EUS) Disease

Cause of disease: EUS is a seasonal disease

Species affected: More on freshwater fish species

(Especially in carps, snake head, etc.)

Bacterial Disease

Vibriosis Disease

Cause of disease: usually occur in the warm summer month

Species affected: Grouper, sea bass, milkfish

Disease prevention and control measure

In Myanmar, the following are the action plans of AAHDCS to prevent and control aquatic animal diseases (Wah & Than, 2016):

a) maintain good water quality and pond environment

b) minimize stress during handling and transportation

c) protect the invasion (prevent the entry) of disease carriers

d) send specimens and report suspected cases to Disease Section

e) collection of fish/shrimp diseases 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 fish/shrimp farmers and students

f) issuance of health certificate after the animal has been examined to be healthy and free from any clinical sign of disease

g) checking of transboundary diseases in live aquatic animals for import or export being performed at Yangon International Airport

Table 1. Levels of disease diagnosis and corresponding activities at the Aquatic Animal Health and Disease Control Section of Myanmar (Wah & Than, 2016)

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
III	Laboratory	Diagnostics (PCR for WSSV, YHV, TSV using the IQ 2000TMKit) and extension service

h) dissemination of pamphlets for aquatic animal disease information and prevention of aquatic animal diseases

i) regular submission of quarterly report on aquatic animal diseases to the Network Aquaculture Centers in Asia-Pacific (NACA) and OIE

Future plan

Aquaculture areas are currently expanding. Consequently, the occurrence of diseases cannot be prevented. Thus, the adherence to good aquaculture practices should be always followed. The AAHDCS of the DOF plays a significant role in controlling and preventing aquatic animal diseases by formulating action plans. Thus, it is important to upgrade laboratory equipment and facilities. Trainings such as the detection and management of aquatic animal diseases should also be conducted.

Training needs and requirements

- A diagnostic laboratory at the Aquatic Animal Health and Disease Control Section in Yangon should be upgraded to meet the requirements of an international standard laboratory.
- The Central Disease Lab is in need of complete renovation.
- Laboratory manuals are not in place for PCR/ Histopathology/ Parasite/ Bacteria.
- The Histology Lab should be set up.
- Trainings on basic diagnosis of parasites, viruses, bacteria and fungi on major culture species such as carps, shrimps, and marine fin fishes should be undertaken.
- It is also necessary to conduct a training on diagnostic capability especially at the Bacteriology and Histology laboratory (Level-II)
- Currently, tests on marine fishes such as grouper and seabass (at Myeik Region) cannot be conducted due to the unavailability to viral test kits.

References

DOF. 2017. Fishery Statistics 2017. Nay Pyi Taw: Department of Fisheries, Republic of the Union of Myanmar Ministry of Livestock, Fisheries and Rural Development.

Regional Technical Consultation on the Aquaculture of *P. vannamei* and Other Exotic Shrimps in Southeast Asia, Manila, Philippines. (2005). Retrieved from https://repository.seafdec.org.ph/handle/10862/847

Saw, N.Y. 2004. Current status of transboundary fish diseases in Myanmar: occurrence, surveillance, research and training, pp. 159-169. *In* C.R. Lavilla-Pitogo & K. Nagasawa (eds.), Transboundary fish diseases in Southeast Asia: occurrence, surveillance, research and training. Proceedings of the Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training, Manila, Philippines, 23-24 June 2004. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Thame, M. & Aye, T.T. 2005. Overview of existing shrimp culture industry and development potential for culture of *P. vannamei* in Myanmar, pp. 57-61. *In* Regional Technical Consultation on the aquaculture of *P. vannamei* and other exotic shrimps in Southeast Asia, Manila, Philippines. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Wah, S.L.P. & Than M.W. 2016. Status of Shrimp Health Management in Myanmar. Retrieved from https://repository.seafdec.org.ph/handle/10862/3091

Status of Aquatic Animal Health in the Philippines

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Abstract

The national aquatic animal disease surveillance and reporting system is implemented by the Bureau of Fisheries and Aquatic Resources in coordination with other recognized laboratories. It covers the OIE/NACA listed diseases particularly those that cause major problems in aquaculture. The fisheries laboratories continuously enhance their capabilities to support the surveillance activities, controls on transboundary movement of aquatic animals, and provide services to the fish farmers. Programs are implemented to strengthen the aquatic animal health services in the country. Promotion of Good Aquaculture Practice and implementation of biosecurity measures are being done to prevent disease occurrences. Collaboration with other institutions on aquatic animal health programs are also established. The paper provides the information on the country's status on aquatic animal health management.

Introduction

The Philippine aquaculture sector contributes about fifty three percent of the country's total fisheries production in 2018 (**Figure 1**). Among the major aquaculture species include tilapia, milkfish, shrimp and its production for the last five years is indicated in **Figure 2** (Philippine Fisheries Profile, 2019). In the same year, the Philippines' has contributed about 0.826 million metric tons of fish, crustaceans and mollusks, equivalent to 1.01 % share to the total global aquaculture production of 82.095 million metric tons, valued over 1.887 billion dollars (FAO Statistics).

The continuous aquaculture intensification increases the risk of disease occurrence and its spread. This has been considered as one of the major hindrance in aquaculture production. Recognizing the impact of diseases and other health problems in fish farming, the implementation of good aquaculture practice and biosecurity measures are important to maximize productivity and ensure safe and quality aquaculture product.

This paper provides the current status of the significant aquatic animal diseases in the Philippines and the programs initiated to control and prevent diseases.

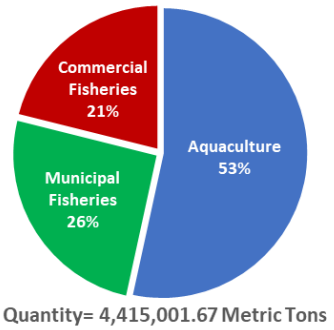

Figure 1. Philippine Fisheries Production CY 2018. Philippine Fisheries Profile, 2019

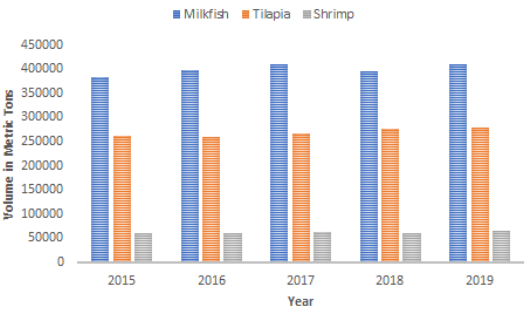

Figure 2. Production data of milkfish, tilapia and shrimp from 2015-2019.

Surveillance system for aquatic animal diseases

Aquatic animal disease surveillance is being conducted by the Bureau of Fisheries and Aquatic Resources. The surveillance activities include those diseases listed in the OIE/NACA. Passive surveillance is implemented at the national level, where reports from the regional fisheries laboratories, and other recognized laboratories are collated at the NFLD for reporting to the OIE/NACA Quarterly Aquatic Animal Disease through the Fisheries Inspection and Quarantine Division. The disease surveillance and reporting system is described in **Figure 3.**

The Department of Agriculture provided the national list of Notifiable Diseases, based on the OIE listed diseases (DA, 2018). The regulations and policies for the controls on transboundary movement of aquatic animals, health certification, biosecurity, disease monitoring, and farm registration geared towards prevention of entry and spread of diseases through issuances of Fisheries Administrative/ Office Orders.

The Philippine National Standards on Code of Good Aquaculture Practices were developed by the Bureau of Agriculture and Fisheries Standards through participative and consultative process with the concerned government agencies and stakeholders. These are basis for the implementation of farm registration scheme for shrimp and fish, on farm inspection and sampling for laboratory analysis. Disease cards regarding significant diseases in aquaculture are being distributed for awareness of the fish farmers. The BFAR central and regional fisheries laboratories and fisheries inspection and quarantine offices involved in the implementation of aquatic animal health management in the country are shown in **Figure 4**.

The laboratory capabilities of the NFLD and regional fisheries laboratories involved in the implementation of aquatic animal health management programs are indicated in **Table 1**. The NFLD as the central laboratory is accredited with PNS ISO/IEC 17025:2017 by the Philippine Accreditation Bureau of the Department of Trade and Industry. The scope of its accreditation includes chemical (residues in fish and fishery products) and biological (microbiological analysis of fish and fishery products and molecular diagnostics) testing, that include the detection of shrimp diseases using conventional PCR.

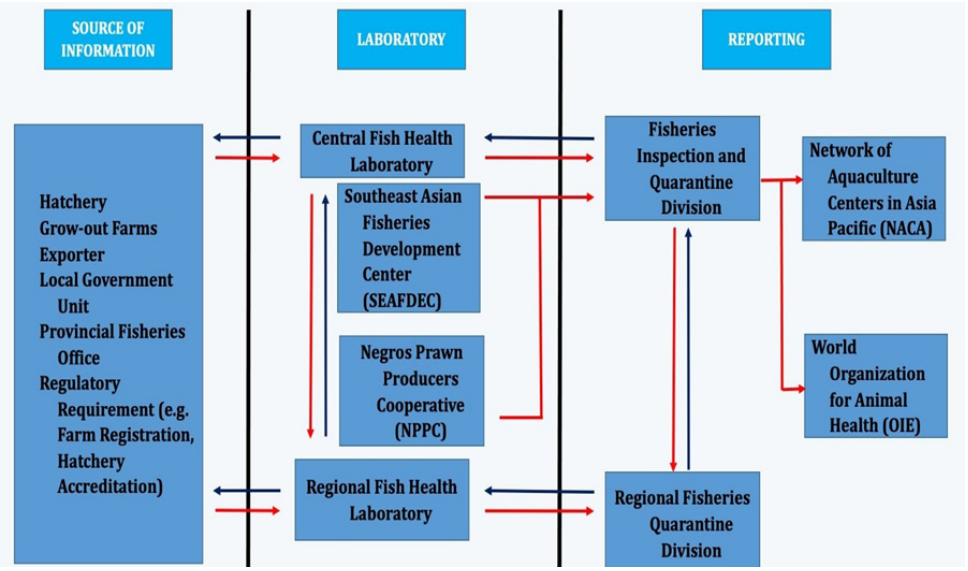

Figure 3. Aquatic animal disease surveillance and reporting system of the Philippines

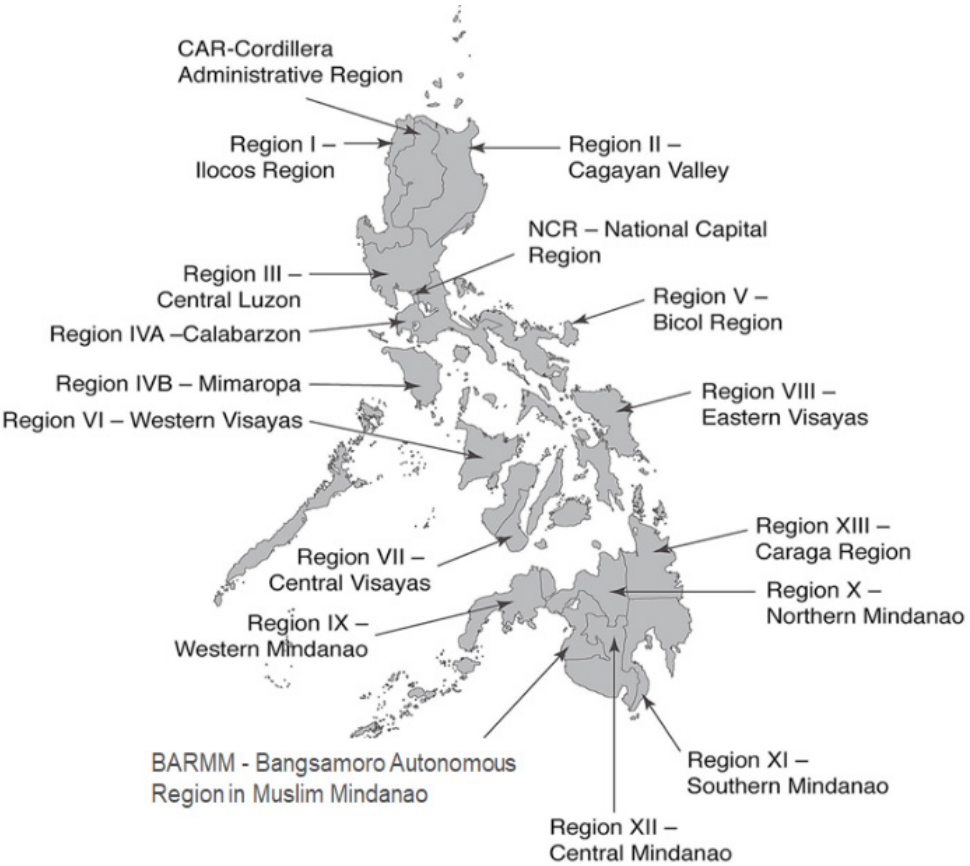

Figure 4. BFAR Central and Regional Fisheries Offices location

The NFLD together with regional fisheries laboratories III and VII participates in the Asia Pacific Laboratory Proficiency Testing for Aquatic Animal Diseases organized by the Australian National Government-Department of Agriculture and Water Resources. The NFLD and the regional fisheries laboratories conducts planning and reporting for a harmonized implementation of programs on aquatic animal health.

The regional fisheries laboratories capabilities depend on the needs of the industry within their areas of their jurisdiction. Regional Fisheries Laboratory VI and VII are also accredited with PNS ISO/IEC 17025:2017 for microbiological and chemical analysis for fish and fishery products. All of the regional fisheries laboratories implement quality management system based on the ISO 17025 standard. They are audited by the NFLD once a year to assess the implementation of various national programs on aquatic animal health including laboratory activities.

Table 1. BFAR laboratories and their level of capabilities on aquatic animal disease detection

BFAR Fisheries Laboratory	Level I	Level II	Level III	
			Screening	Confirmatory
NFLD	√	√	√	√*
NCR	√	√	√	-
I	√	√	√	-
II	√	√	√	√
III	√	√	√	√
IV-A	√	√	√	-
MIMAROPA	√	√	√	-
V	√	√	√	-
VI	√	√	√	√*
VII	√	√	√	√*
VIII	√	√	√	-
IX	√	√	√	-
X	√	√	√	-
XI	√	√	√	-
XII	√	√	√	-
XIII	√	√	√	-
CAR	√	√	√	-
CARAGA	√	√	√	√
BARMM	√	√	√	-

Level I - Observation of animal and environment, Gross clinical examination;
Level II - Parasitology, Bacteriology, Histopathology;
Level III - Molecular biology, Immunology (i. Screening - Insulated Isothermal Polymerase Chain Reaction (iiPCR)/ POCKIT or POCKIT microPlus, ii. Confirmatory - Conventional PCR*, Real-time PCR)

Reference: Asia Diagnostic Guide to Aquatic Animal Diseases (eds. Melba G. Bondad-Reantaso, Sharon E. McGladdery, Iain East and Rohana P. Subasinghe). FAO Fisheries Technical Paper 402-2. Rome* PNS/IEC ISO 17025:2017 Accredited

Aquatic animal diseases

Diseases in shrimp

White Spot Disease (WSD)

WSD is considered as one of the major diseases of shrimp that causes massive mortalities. In the Philippines, WSD in *Penaeus monodon* was first detected from surveillance activities and reported to the Network of Aquaculture Centres in Asia-Pacific (NACA) in January 1999 (NACA/FAO QAAD, 1999). The disease was also detected in various geographic locations such as Agusan del Norte, Bataan, Batangas, Bulacan, Camarines Norte, Cebu, Negros Occidental, Oriental Mindoro, Quezon, Sarangani Province and Zamboanga del Sur in January to May of the same year (Tapay *et. al.*, 2000).

Mass mortalities due to WSD have been experienced in major shrimp producing areas in the country since 2002 affecting shrimps of about 60–90 days of culture. The disease caused 80 to 95 % mortality in intensive culture systems, while 30 to 70 % in extensive culture systems (de la Pena *et al*., 2007). At present, WSD are now detected by BFAR laboratories at post-larvae up to adult stages without seasonal pattern. High mortalities are observed in some cases and emergency harvest of stocks are being done in harvestable sizes whenever possible.

Mixed infection with other shrimp diseases can be observed in some cases. Result of WSSV surveillance of BFAR from 2015–2019 is shown in **Figure 5**.

WSD can cause rapid mass mortality, lethargy, cessation of feeding and moribund animals are observed floating at the edge of the pond. Grossly, infected animals commonly exhibit loosened carapace, reddish to pinkish discoloration of the body and appendage and white calcium deposits embedded in the shell. Histopathological examination shows inclusion bodies and hypertrophied nuclei in cuticular epithelium and gills. A reported case of WSSV infection with clinical signs and histopathogical examination is shown in **Figures 6** and **7**.

Acute hepatopancreatic necrosis disease (AHPND)

AHPND was first detected in the Philippines in *Penaeus vannamei* and *Penaeus monodon* in the provinces of Bataan, Bulacan and Pampanga in Central Luzon 2014 (NACA/FAO QAAD, 2014; Dabu *et al*., 2015; de la Peña *et al*., 2015). Species affected are *Penaeus vannamei*, *P. monodon*, *P. merguiensis*, and *P. indicus*. The disease can affect all stages of the culture period (Apostol-Albaladejo, 2016). Mortalities occur within 0–35 days and as early as 10 days of stocking shrimp postlarvae or juveniles. However, the disease is also observed to occur as late as 46–96 after stocking (de la Peña *et al*., 2015). Among the clinical signs observed include inappetence, empty hepatopancreas and stomach, rubbery textured hepatopancreas, in some cases with white feces, and rapid mortality. **Figures 8** and **9** shows a shrimp with pale hepatopancreas, and histopathological examination of hepatopancreas with sloughing epithelial tubules, respectively

Shrimps infected with AHPND manifests several degrees of pathological changes in the hepatopancreas such as loosening and minimal degradation of tubule epithelial cells in acute phase. In early phase of infection, there is sloughing around the tubule leading to greater space, and at the terminal stage of infection, others manifest enlargement of the nuclei and presence of bacterial colonies may be observed in

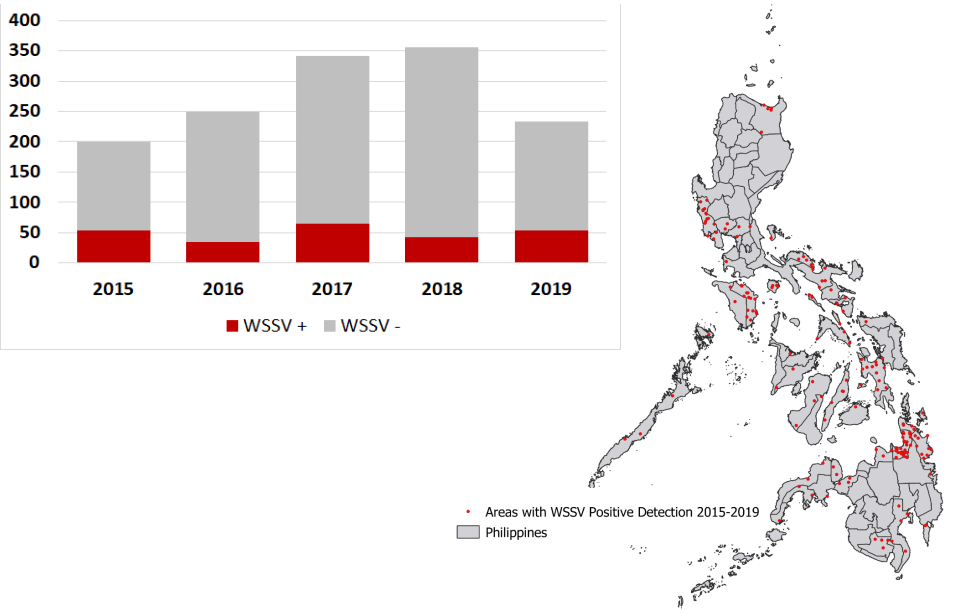

Figure 5. Graph and map showing WSSV detection from surveillance activities (2015-2019)

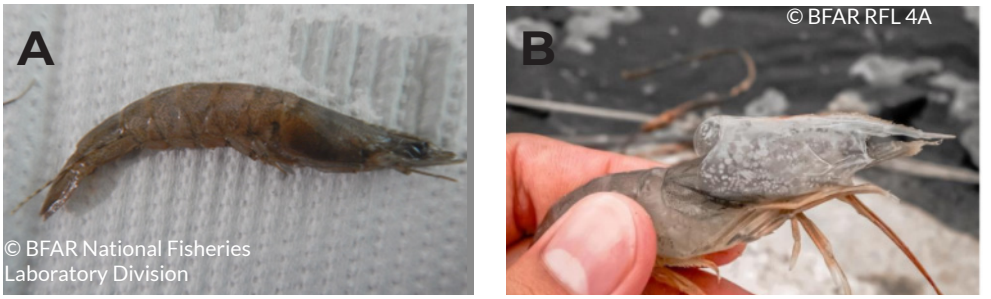

Figure 6. Shrimp with signs of WSSV infection, a) reddish discoloration of the body ; b) calcium deposits in carapace.

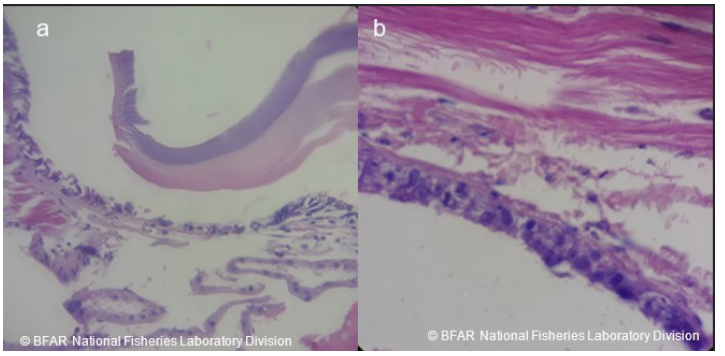

Figure 7. Histopathological examination of *P. vannamei* showing **a)** disintegration of epithelial lining of the mouth (H&E staining- 400x), and **b)** presence of intranuclear inclusion bodies and nuclear pyknosis (H&E staining- 1000x).

hepatopancreas tubule epithelial cells (Dabu *et al*., 2015).

The BFAR surveillance activities for AHPND from 2015–2019 is shown in **Figure 10.** Based on the results of the surveillance, the BFAR laboratories detected the disease on a wide range of shrimp culture stages from post-larvae to adult. Detection by the laboratory did not provide any seasonal pattern on its occurrence. However, mortalities are more common at post-larvae to juvenile stage. Currently, infection by AHPND can be observed to be associated with other diseases such as IHHNV, WSSV and EHP.

Hepatopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei (HPM-EHP)

HPM-EHP is an emerging disease of shrimp caused by a fungal microsporidian parasite that infects the hepatopancreas. In the Philippines, EHP was detected in various shrimp species including *P. vannamei*, *P. monodon*, *Penaeus indicus* and *Penaeus merguensis*. It was first detected in *P. vannamei* from Cebu by the BFAR laboratory in 2016 (NACA/FAO QAAD, 2016).

The disease signs include growth retardation, uneven sizes and some may

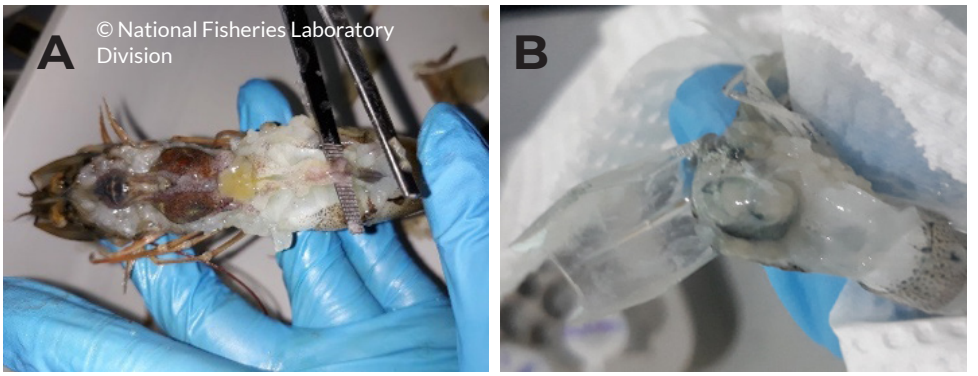

Figure 8. Comparison of shrimp with (a) normal hepatopancreas (b) pale hepatopancreas with AHPND

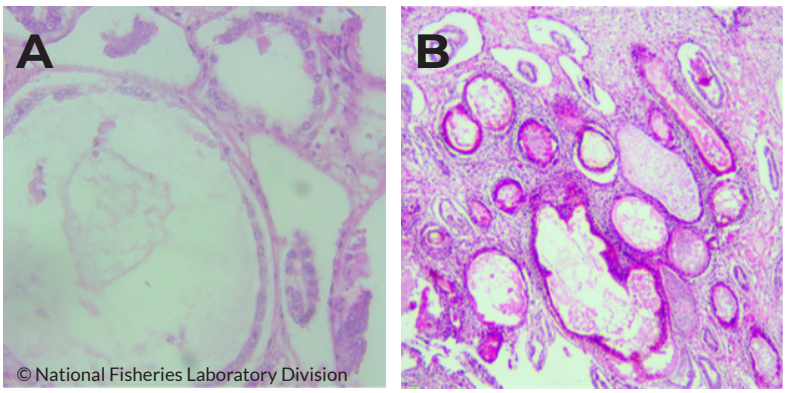

Figure 9. Histopathological examination of *P. vannamei* with AHPND showing **a)** rounding and sloughing off of hepatopancreas tubule epithelial cells and **b)** inflammation of hepatopancreas (H&E staining-1000x)

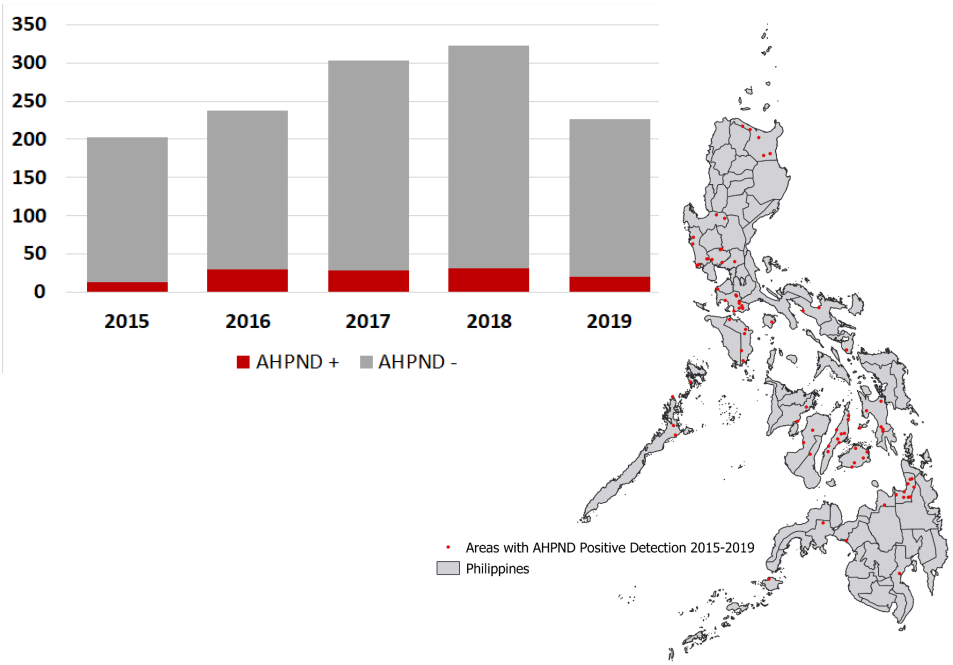

Figure 10. Graph shows the number of farms with detection of AHPND and total number of farms monitored from 2015-2019. The Map shows location and distribution of the disease in the Philippines

exhibit soft body/segment. No significant mortalities were observed in EHP infected animals even in co-infection cases with IHHNV. Mortalities are experienced in mixed infection with WSD and AHPND. Pitogo (2016) provided recommended disinfection measures to minimize EHP from maturation facilities, hatcheries, and grow-out farms using hydrated lime or hydrochloric acid before stocking and implementing quality control in post-larvae selection. A reported case of mixed EHP and AHPND infection is shown in **Figure 11**. The surveillance activity of BFAR on EHP from 2016–2019 is presented in **Figure 12.**

Other shrimp diseases

Other diseases of shrimp in the Philippines include infection with Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Baculovirosis and Yellow-Head Disease. IHHNV affects all life stages of several species such as *P. vannamei*, *P. monodon*, *P. stylirostris*, *P. merguiensis* and *P. indicus*. It was first reported in March 2006, on postlarvae *Penaeus vannamei* from Zambales conducted by SEAFDEC-AQD (NACA/FAO QAAD, 2006). Common disease signs may include cannibalism, poor hatching of eggs and poor survival of larvae and PL, irregular growth rate and deformities.

Monodon Baculovirus (MBV), is the first diagnosed viral disease in the Philippines in 1989–1990 in all 12 shrimp producing provinces. All life stages of *P. monodon* are affected. Spherical, eosinophilic occlusion bodies fill up enlarged nuclei of hepatopancreatic cells and are discharged into the lumen under microscope. Elimination of fecal contamination of spawned eggs and larvae by thoroughly washing nauplii or eggs with formalin, iodophors and clean sea water.

Figure 11. Shrimp with mixed infection of EHP and AHPND shows uneven sizes with reddish discoloration

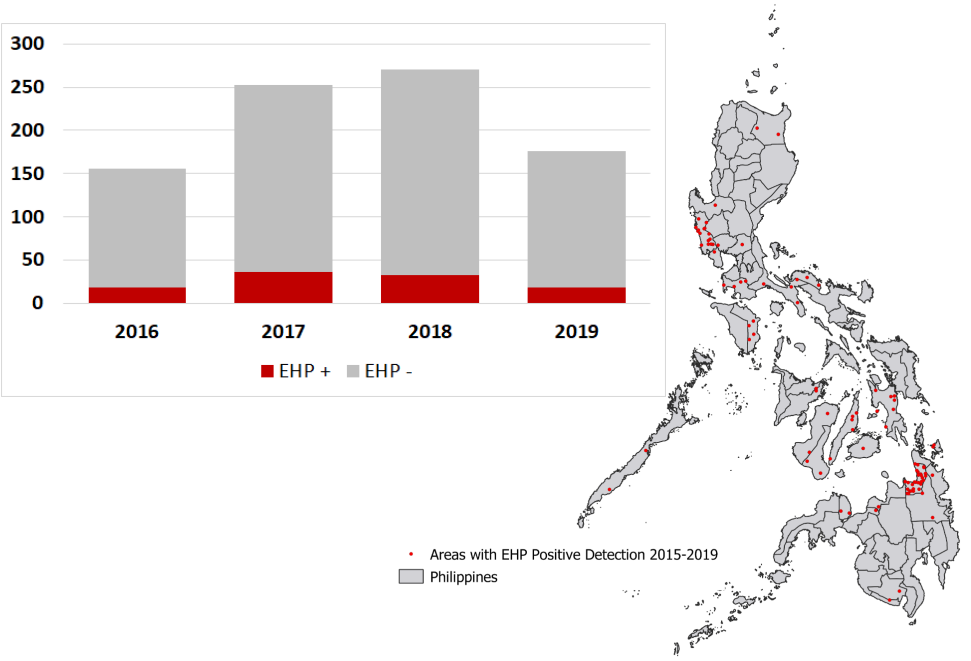

Figure 12. Graph demonstrating the number of samples and detection of EHP in shrimp. The map shows the areas with detection of EHP (2016-2019)

Yellow Head Disease caused by yellow head virus (YHV) was detected in 1998 by Albaladejo *et al*. Affected shrimp population was observed to have poor growth performance and with mortalities from 40–80 %. The reported case showed mixed infection with Luminous bacteria and spawner-isolated mortality virus (SMV) in some samples. The last occurrence in the Philippines was reported in July 1999, and have not been detected until the present time. In previous years, the luminous bacteria was a significant problem in shrimp culture. Polyculture system of shrimp with finfish or oyster/ mussels can control the growth of luminous bacteria (Tendencia, 2007).

Viral diseases

Tilapia Lake Virus (TiLV)

In the Philippines, Tilapia Lake Virus (TiLV), or Syncytial Hepatitis of Tilapia (SHT), was first detected in tilapia (*Oreochromis niloticus*) in the province of Bulacan in May 2017 and reported to the OIE in the same year. The first occurrence happened in a tilapia nursery farm wherein fingerlings showed daily mortality that reached up to 25 % after 15 days of culture. The infected fish have demonstrated skin erosions and discoloration (darkening), distended abdomen, scale protrusion, exophthalmia, and paleness of the gills. TiLV infection in tilapia fingerlings is shown in **Figure 13**.

Figure 13. Tilapia fry showing distended abdomen and exophthalmia

The disease is observed during summer up to the onset of the rainy seasons from April to June. Some samples tested positive in iiPCR however typical signs of the disease are not apparent. Since its first occurrence, surveillance activity has continued on a national scale (**Figure 14**).

Currently, a collaborative project is being undertaken by the SEAFDEC/AQD Binangonan Freshwater Station with BFAR on detection, quantification, and viability of Tilapia Lake Virus (TiLV) in pond soil and water as influenced by water quality parameters and culture management.

Viral nervous necrosis (VNN)

Viral nervous necrosis (VNN), also known as Viral encephalopathy and retinopathy (VER) causes mass mortalities in marine fishes such seabass (*Lates calcalifer*) larvae and grouper (*Epinephelus coioides)* (de la Pena *et al*., 2008). The disease is caused by Betanodavirus and the infected fish exhibits abnormal swimming behavior, anorexia, lethargy, and some develops skin lesion. The organ most affected for VNN is the brain and optic nerves. In addition, the diseases can be observed in mixed infections with other pathogen

Irido Megalocytivirus

Irido Megalocytivirus is being detected in groupers (*Epinephelus* spp.), in juveniles to grow out stages. Farms positive to the disease experienced massive mortalities. Clinical signs include lethargy, uncoordinated swimming, and distended abdomen. Internal examination showed signs of spleen enlargement and it also affects the kidney.

Koi Herpesvirus

Koi Herpesvirus (KHV) is included in the surveillance activity of BFAR. Up to present time, Philippines is KHV-free country. In 2010, the first case of KHV associated mortality was from illegally imported koi carp bought by a passenger from an ornamental fish show in China, but its origin was unknown. It was confiscated by the Fisheries Quarantine and Inspection Service Officers at the Ninoy Aquino International Airport (NAIA). Five days after confiscation, fish showed symptoms such as necrotic gill filaments, body ulcerations, discolored patches and pale body coloration. The disease was confirmed by PCR analysis (Somga *et al*., 2010). The disease has not been detected locally in surveillance activities of BFAR and conform with the study conducted by Lio-Po *et al*. 2009.

Bacterial diseases

Streptococcosis

Streptococcosis in the Philippines is mainly caused by *Streptococcus agalactiae* and *S. iniae*. These are associated with highly contagious and significant mortalities in tilapia farming, specifically in grow-out cage culture in Taal Lake, Batangas. Locals refer to disease as "hibay" wherein affected fish demonstrate scale loss, fin rot, lethargy, abnormal swimming behavior such as swirling and loss of balance. Terminal stage of the disease presents abdominal distention, exophthalmia, and hemorrhages in the different parts of the body (**Figure 15)**. Pathological changes in the internal organs can be observed such as enlargement of liver, spleen, kidney and gallbladder, petechiae and focal necrosis of liver, and hemorrhages in the brain. The moribund fish are fragile and have a foul smelling odor.

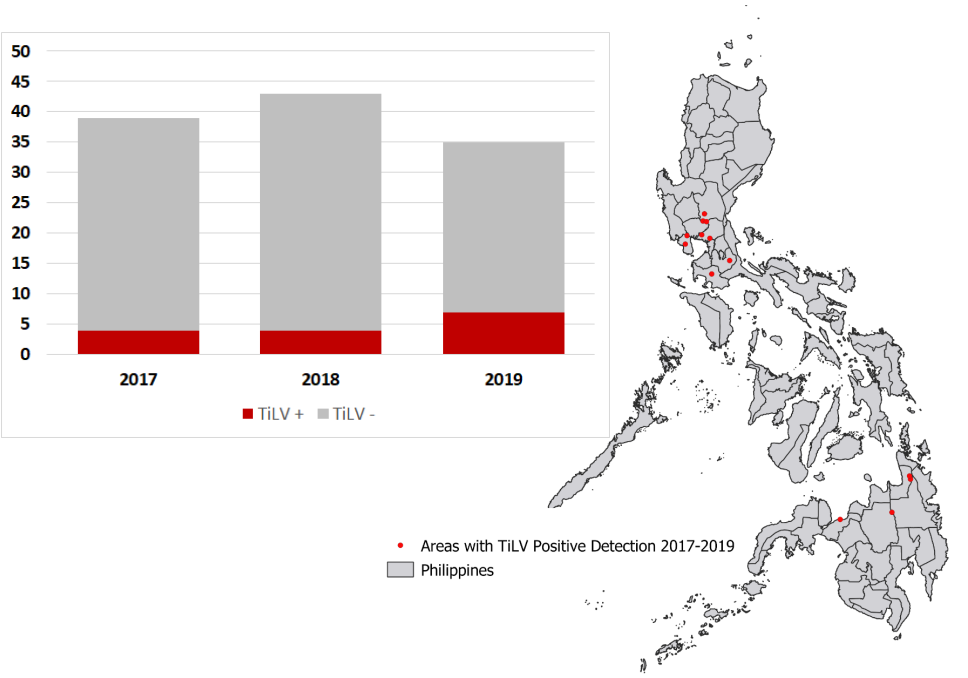

Figure 14. Graph showing positive detection with TiLV from the total number of samples analyzed. The areas with detection of TiLV (2017–2019)

Aeromonas Infection

Aeromonas infections in tilapia, caused by *Aeromonas hydrophila*, are also experienced by farmers as a result of any stressor to the animal such as abrupt changes in the temperature, overstocking, and poor water quality. Clinical signs and patterns of mortality are similar to other common bacterial infections. Pakingking *et al*, (2015) studied the composition of bacterial microbiota in the rearing water, sediment, gills and intestines in tilapia that could cause disease in stressful conditions.

Vibriosis

Vibriosis in marine fish is the most common bacterial infection and most cases are chronic and may affect all fish stocks. The disease is characterized by hemorrhagic ulcers penetrating muscle tissue (**Figure 16)**. The affected fish show hemorrhages in fins, tail rot, cloudiness of the eye may occur at the advance stage of the disease. It is also associated with heavy parasite infestation particularly in small fishes.

Nocardiosis

Nocardiosis caused by *Nocardia* spp. has been detected in cage-cultured pompano in at Padre Burgos, Quezon, Philippines. The morbidity rate was about 80–100 % while the mortality rate was estimated at 50–60 %. Clinical signs of the disease were lethargy, emaciation, loss of scales, skin ulcers on the body and whitish to yellowish green, irregularly shaped masses at the base of the gill filaments. Internally, nodules in the liver, spleen and kidney were seen (**Figure 17**). Histopathological examination showed multiple granulomas in the kidney, liver, spleen and muscle.

Figure 15. Tilapia samples taken from affected population with bugling eyeball and distended abdomen

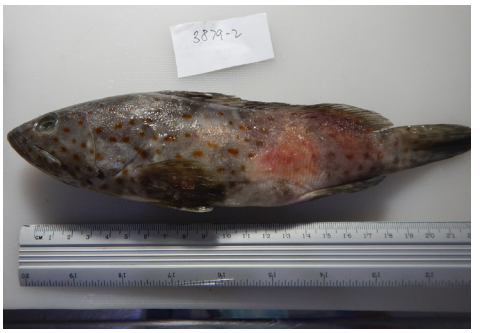

Figure 16. Grouper with clinical signs of bacterial infection.

Epizootic Ulcerative Syndrome (EUS)

Epizootic Ulcerative Syndrome (EUS) caused by *Aphanomyces invadans* first confirmed occurrence in the Philippines was in late 1985 to early 1986 at Laguna Lake affecting bottom dwelling species like snakehead (*Ophicephalus striatus*), catfish

(*Clarias batrachus*), gouramy (*Trichogaster pectoralis*), goby (*Glossobius giurus*), crucian carp (*Carassius carassius*), Manila sea catfish (*Arius manillensis*), and silvery theraponid (*Therapon plumbius*) (Llobera and Gacutan, 1987). The last report of EUS was in October 2002 in African sharptooth catfish from Sta. Barbara, Iloilo. It is only in January to April 2019 that the disease is reported to have re-occurred in gobies (*Awaous* spp.) in San Juan river, Abra. The disease usually occurs during cold months causing shallow to deep ulcerations from the different parts of the body. Risk factors for EUS infection includes low-water temperature, low alkalinity, low hardness and chloride, fluctuating pH and heavy rainfall (Bondad-Reantaso *et al*., 1992). Histopathology reveals muscle degeneration and necrosis with fungal hyphae and mycotic granuloma formation using grocott's methenamine silver stain (**Figure 18**). EUS is difficult to control as most cases occur in wild populations.

Microsporidiosis

Microsporidiosis is caused by microsporidians, *Glugea* spp. and *Pleistophora* spp.,(Nagasawa and Lacierda 2004). Reported cases of microsporidiosis were seen in groupers (*Epinephelus* spp. and *Cromileptes* spp.). Clinical signs include abdominal swelling, and presence of dark brown cyst-like formed in fat tissues and internal organs (**Figure 19**). It affects nursery and grow-out stages. Mortality rate in affected stocks is variable.

Figure 17. a) pompano showing loss of scales, emaciation and skin ulcers (red arrow), **b)** pompano gills with irregularly shaped masses at the base of gill filaments, **c)** liver with nodules, and **d)** pleen with nodules

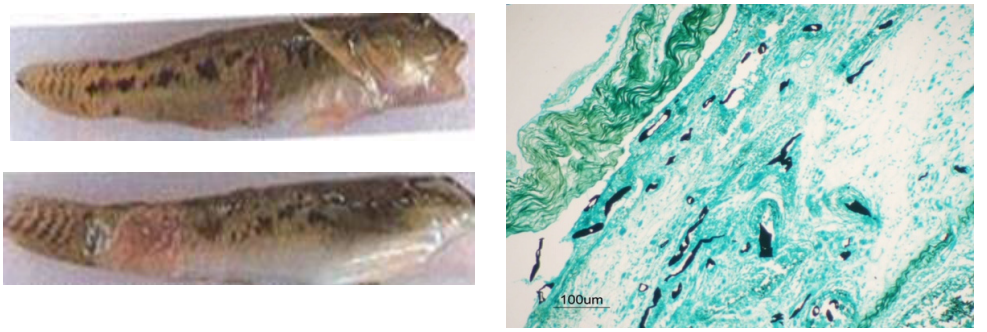

Figure 18. Goby infected with EUS, and histopathological examination of muscle tissue showed presence of fungal hyphae (Grocott's Methenamine silver stain, 10X)

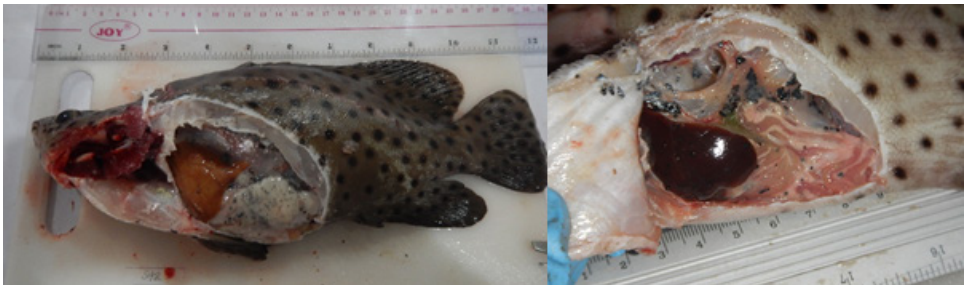

Figure 19. Panther Grouper infected with microsporidiosis

Common parasites in finfish

Common protozoan parasites in freshwater fishes include *Trichodina* spp. and *Ichthyopthirius multifiliis*. These are the most prevalent parasites in fish (Natividad *et al*., 1986; Bondad-Reantaso and Arthur, 1989). The BFAR laboratory commonly detects *Trichodina* parasites in eels, carp, and tilapia fry and fingerlings intended for local movement and export. On the other hand, *Ichthyopthirius multifiliis* or "White Spot Disease '' is detected in tilapia imported from other countries (**Figure 20**). These parasites can cause discomfort, skin and gill damage, respiratory distress, and predisposes fish to opportunistic organisms.

Other parasites commonly detected in fishes are the skin fluke, *Dactylogyrus* spp. and gill fluke, *Gyrodactylus* spp. (**Figure 21**). Some of the signs and symptoms of infestation in fish include swollen and pale gills, loss of scale and a change in color in areas where the parasites are attached and lethargy (Cruz-Lacierda, 2010). The gill epithelium may be severely damaged affecting normal respiration. Heavy infestations may cause high mortalities. Most commonly affected are at fingerlings stage.

Disease control measures

There are several good aquaculture practices recommended for disease control and prevention (PNS, 2014). The GaqP covers elements on food safety, animal health and welfare, environmental integrity and social aspects. Specific farm management practices against significant diseases in shrimp are also available for the stakeholders such as acute hepatopancreatic necrosis disease

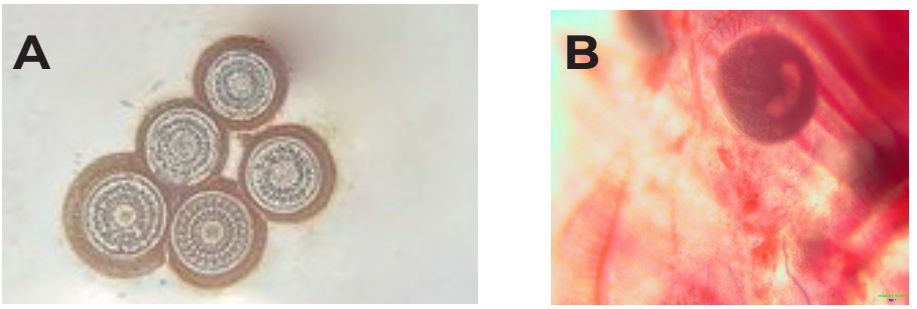

Figure 20. a) *Trichodina* spp. isolated from tilapia fingerling samples, and **b)** *Ichthyopthirius multifiliis* isolated from grass carp (10X)

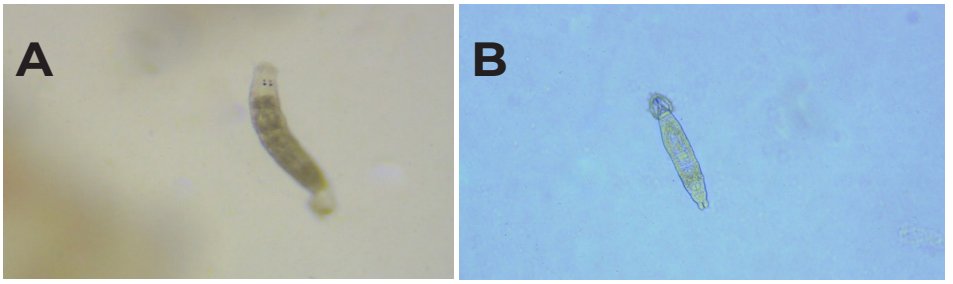

Figure 21. (a) Skin fluke and, **(b)** gill fluke isolated from tilapia (40X)

(Usero and Apostol-Albaladejo, 2015). The DOST-PCAARD also published handbooks on the Philippine Recommends of different aquatic animal species such as tilapia and milkfish. The handbooks described techniques and provide valuable information in the management and disease control measures in every stage of the rearing process. There are also disease recognition cards for significant diseases by BFAR which also provides various disease control measures.

Cooperation on the aquatic animal health management programs

The aquatic animal health services of the Philippines were evaluated by the OIE experts using the OIE Aquatic PVS Tool on 3–18 Feb. 2013. It was aimed to identify strength and weakness of the aquatic animal health services in compliance with the criteria set out in the OIE aquatic animal health code in particular quality of aquatic animal health services. It includes assessment of the human, physical and financial resources, capabilities, interaction with interested parties and access to markets. Recommendations on how to improve the aquatic animal health services were provided (Somga *et al*., 2015). In 18–28 Jan. 2016, another OIE experts mission conducted Gap Analysis in the implementation of priority programs on aquatic animal health services, to determine the level of performance and gaps that needs to be strengthened. Short to medium term plan of activities were developed to achieve the goals set in improving aquatic animal health services. Continuous strengthening of aquatic animal health services is being done.

There are also several projects being implemented by BFAR on aquatic animal health in collaboration with other international organization such as the

Food and Agriculture Organization (FAO), World Organization for Animal Health Office (OIE), Network of Aquaculture Center in the Asia Pacific (NACA), Southeast Asian Fisheries Development Center (SEAFDEC), and other research institutes.

Acknowledgement

We would like to thank all the Regional Fish Health Officers for the data used in this paper. Also, we would like to express our gratitude to SEAFDEC for the opportunity to participate in this project.

References

Albaladejo, J. D., Tapay, L. M., Migo, V. P., Alfafara, C. G., Somga, J. R., Mayo, S. L., Miranda, R. C., Natividad, K., Magbanua, F. O., Itami, T., Matsumura, M., Nadala, E. C. B. Jr. & Loh, P. C. 1998. Screening for shrimp viruses in the Philippines. In: Advances in Shrimp Biotechnology, Flegel, T. W., ed. National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand, 251-253.

Apostol-Albaladejo, M.G. 2016. Status of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimps in the Philippines. In Proceedings of the ASEAN Regional technical consultation on EMS/AHPND and other transboundary diseases of improved aquatic animals in Southeast Asia, Pakingking, R.V., deJesus-Ayson E.G.T, Acosta B., eds., 22-24 February 2016, Makati City Philippines. SEAFDEC. 65-72.

BAFS. 2014. PNS/BAFS 135:2014. Code of Good Aquaculture Practice. ICS 65.150. 28pp

Bondad-Reantaso, M.G. & Arthur, J.R. 1989. Trichodinids (Protozoa: Ciliophora: Peritrichida) of Nile tilapia (*Oreochromis niloticus*) in the Philippines. Asian Fisheries Science

Bondad-Reantaso, M. G., East, I.J., Subasinghe, R. 2001. Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fisheries Technical Paper Number 402-2. Food and Agriculture Organization of the United Nations.

Bondad-Reantaso, M.G., Lumanlan, S.C., Natividad, J.M., Phillips, M.J. 1992. Environmental monitoring of the epizootic ulcerative syndrome (EUS) in fish from Munoz, Nueva Ecija, Philippines, in: Shariff, I.M., Subsasinghe, R.P., Arthur, J.R. (Eds.), Diseases in Asian Aquaculture. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 475–490.

Cruz-Lacierda, E. 2010. "Parasitic Diseases and Pests" Health Management in Aquaculture Second Edition. Aquaculture Department. Southeast Asian Fisheries Development Center.

Dabu, I., Lim, J.J, Arabit, P.M., Orense, S.J., Tabardillo Jr., J., Corre Jr., V., Manigas, M.B. 2015. The first record of acute hepatopancreatic necrosis disease in the Philippines. Aquacult. Res. 1-8. doi: 10.1111/are.12923.

De la Peña L. D., Lavilla-Pitogo C. R., Villar C. B. R., Paner M. G., Capulos G. C. 2007. Prevalence of white spot syndrome (WSSV) in wild shrimp *Penaeus monodon* in the Philippines. Diseases of Aquatic Organisms. Vol. 77:175-179

De la Pena, L.D., Mori K., Quinitio G.F., Chavez D.S., Toledo J.D., Suarnaba V.S., Maeno Y, Kiryu I. and Nakai T. 2008. Characterization of betanodaviruses in the Philippines. Bull. Eur. Ass. Fish Pathology 28(6): 230-237.

De la Peña, L. D., Cabillon, N.A.R., Catedral, D.D., Amar, E.C., Usero, R.C., Monotilla, W.D., Calpe, A.T., Fernandez, D.D.G. Saloma, C.P. 2015. Acute hepatopancreatic necrosis disease (AHPND) Outbreaks in *Penaeus vanamei* and *P. monodon* cultured in the Philippines. Diseases of Aquatic Organisms 116: 251-254.

Department of Agriculture. 2018. Amendment to DA administrative order No. 01, series of 2012 on declaring the list of notifiable animal diseases. Retrieved from: https://www.da.gov.ph/wp-content/uploads/2018/05/ac03_s2018.pdf?fbclid=IwAR2F86ZKIJW2qgZmTjWj2GDJaW6sXmDyj_Zl02whKdnknMrLyuYnMVw-9S8

FAO. 2018. Food and Agriculture Organization of the United Nations. FAO Statistics. Fishery and Aquaculture Statistics 2018.

Llobrera A.T., Gacutan R.Q. 1987. *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. Aquaculture 67:273-278

Maria, P.I., Po, G.L. 2010. Koi Herpesvirus-associated mortalities in quarantines koi carp in the Philippines. Bull. Eur. Ass. Fish Pathology 30(1) 2-7.

Miranda, R. O., Albadejo, J. D., Nadala, E. C. B. Jr., Loh P. C. 2000. White spot syndrome virus (WSSV) in cultured *Penaeus monodon* in the Philippines. Dis Aquat Org 42:77-82.

Nagasawa, K., & Cruz-Lacierda, E. R. (Eds.). 2004. Diseases of cultured groupers. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Natividad J.M., Reantaso M.G.B., Arthur J.R. 1986. Parasites of Nile Tilapia (*Oreochromis niloticus*) in the Philippines. Bureau of Fisheries and Aquatic Resources, Quezon City (Philippines)

Network of Aquaculture Centres in Asia-Pacific and Food and Agriculture Organization of the United Nations. 1999. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 99/1, January-March 1999. FAO Project TCP/RAS/6714. Bangkok, Thailand

Network of Aquaculture Centres in Asia-Pacific and Food and Agriculture Organization of the United Nations. 2006. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 2006/1, January-March 2006. FAO Project TCP/RAS/6714. Bangkok, Thailand

Network of Aquaculture Centres in Asia-Pacific and Food and Agriculture Organization of the United Nations. 2014. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 2014/3, July- September 2014. FAO Project TCP/RAS/6714. Bangkok, Thailand

Network of Aquaculture Centres in Asia-Pacific and Food and Agriculture Organization of the United Nations. 2016. Quarterly Aquatic Animal Disease Report (Asia and Pacific Region), 2016/2, April-June 2016. FAO Project TCP/RAS/6714. Bangkok, Thailand

Pakingking JR., Palma R and Usero R. 2015. Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthern ponds in the Philippines. World Journal of Microbiology and Biotechnology, 31(2). 265-275.

Philippine Fisheries Profile. 2019. Bureau of Fisheries and Aquatic Resources. ISSN: 2704-3355

Pitogo, Celia L. 2016. "Understanding the microsporidian parasite, *Enterocytozoon hepatopenaei (EHP)*, and its implications on shrimp hatchery and farm management. Aquaculture Department. Southeast Asian Fisheries Development Center.

Somga, J. R., de la Pena LD, Sombito C.D., Paner M.G., Suarnaba V.S., Capulos G.C., Santa

Somga, J., Regidor S.,Somga S., and Balagapo R. 2016. The Philippines' experience with the OIE PVS evaluation. In: Proceedings of the 3rd OIE Global Conference on Aquatic Animal Health: Aquatic Animal Health: 'Riding the wave to the future. Ho Chi Minh City Vietnam, 20-22 January 2015. OIE.

Tapay, L. M., Magbanua, F. O., Natividad, K. T., Migo, V. P., Alfafara, C. G., De la Peña, F. O.,

Tendencia, E.A. 2007. Polyculture of green mussels, brown mussels and oyster with shrimp control luminous bacterial disease in a simulated culture system. Aquaculture. 272. 188-191.

The Milkfish Technical Committee. 2016. The Philippines recommends for milkfish. Los Baños, Laguna, Philippines: DOST-PCAARRD.

The Tilapia Technical Committee. 2018. The Philippines recommends for tilapia. Los Baños, Laguna, Philippines: DOST-PCAARRD.

Usero R., and M.A.G. Apostol-Albaladejo. 2015. Philippine shrimp grow-out farm management practices against acute hepatopancreatic Necrosis Disease (AHPND) with emphasis on green water technology. Bureau of Fisheries and Aquatic Resources (BFAR) and Negros Prawn Producers Cooperative (NPPC)

Country Report - Singapore

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Abstract

The aquaculture industry produces about 10 % of Singapore's annual local fish consumption. By 2030, the country's goal is for the agri-food industry to produce 30 % of Singapore's nutritional needs. In order to achieve this, the Marine Aquaculture Centre (MAC) spearheaded several research and development programmes such as the broodstock development of Asian seabass and large-scale fry production technology. MAC also provides technical support to local farms to adopt sustainable farming practices and technology. In 2019, the Singapore Food Agency (SFA) was formed to oversee all matters pertaining to food supply and safety, including seafood production from aquaculture. The National Centre for Food Safety (NCFS) of SFA and Centre for Animal and Veterinary Services (CAVS) under National Parks Board (NParks) provides support via their diagnostic and testing capabilities in the areas of food safety and animal health, respectively.

Based on the national surveillance program conducted by the SFA and NParks, the commonly reported diseases are viral, bacterial or parasitic infections. These include Benedeniosis, Big-Belly (BB) Disease Syndrome, infections with *Streptococcus iniae* and *Tenacibaculum maritimum*, *Norcardiosis*, Viral Nervous Necrosis (VNN), and Infectious Spleen and Kidney Necrosis Virus (ISKNV). With the aim to control and manage the aquatic diseases in Singapore, control measures for major pathogens in fin fishes and crustaceans are being implemented. In addition, Aquatic Animal Health Professionals (AAHP) should undergo trainings in aquaculture farm biosecurity.

Status of aquaculture in the country

Singapore is a small country state (land area of 719.9 km^2) with a demographic profile of 5.6 million in population (Yearbook of statistics Singapore, 2018). Aquaculture in Singapore consists of both the food and ornamental fish industry. As of 2017, there are 117 coastal fish farms, 9 land-based food fish farms and 60 ornamental aquarium fish farms licensed by the former Agri-Food & Veterinary Authority (AVA).

The food fish aquaculture industry produces about 10 % of Singapore's annual local fish consumption. We aim to develop the capability & capacity of our agri-food industry to produce 30 % of Singapore's nutritional needs by 2030.

The Singapore Food Agency (SFA) was formed as a new statutory board under the Ministry of the Environment and Water Resources on 1 April 2019. The SFA brings together food-related functions carried out by the former Agri-Food & Veterinary

Authority of Singapore, the National Environment Agency and the Health Sciences Authority.

Culture systems and extent of culture

Marine food fish are cultured on mainly offshore coastal fish farms located along the Johor Straits. Most coastal farms adopt shallow wooden-caged designs, and the nets typically measure 3 x 3 x 2.5 or 5 x 3 x 2.5 meters. Some have longer, extended nets to facilitate maintenance – this allows different portions of the net to be regularly raised for drying out, thus reducing fouling by marine organisms. Some coastal farms along the Johor Straits adopted closed containment systems, which separate the water where the fish are kept from the water in the natural environment. These can be tanks placed in barges or wooden platforms. Seawater is treated before entering the system and then discharged without being reused. During adverse environmental conditions (e.g., harmful algae bloom), the system can be operated in a recirculation mode which the water from the containment system is treated and reused. There is also one open-sea farm off the Southern Islands of Singapore using high-density polyethylene (HDPE) circular floating cages. As for land-based fish farms, most farms use traditional culture systems such as mud-based ponds, concrete tanks and fibreglass/ glass tanks, while some farms use multi-storey Recirculating Aquaculture Systems (RAS) to produce fish and shrimp.

Species farmed and production trends

The floating farms in Singapore culture a variety of high-value food finfish for local consumption and for export purposes. The main species of marine food finfish cultured are the Asian sea bass (*Lates calcarifer*), groupers (*Epinephelus* spp.), milkfish (*Chanos chanos*), mullet (*Mugil cephalus*), long dorsal fin pompano (*Trachinotus blochii)*, snappers (*Lutjanus* spp.), four finger threadfin (*Eleutheronema tetradactylum*) and golden trevally (*Gnathanodon speciosus*). The fish are purchased as fry from either local hatcheries or imported from neighbouring countries.

Ornamental fish produced in Singapore are solely freshwater species. This includes the live-bearers: Guppy, Platy, Swordtail and

Table 1. Farm production of food fish and aquarium fish for the last 7 years

Year	2011	2012	2013	2014	2015	2016	2017
Food Fish (tonnes)	5,094	5,127	5,864	5,639	6,540	6,086	5,916
Aquarium Fish (mil pcs)	110	106	114	109	77	77	73

[1]The Singapore Food Agency (SFA) was formed as a new statutory board under the Ministry of the Environment and Water Resources on 1 April 2019. The SFA brings together food-related functions carried out by the former Agri-Food & Veterinary Authority of Singapore, the National Environment Agency and the Health Sciences Authority.

Source: https://www.singstat.gov.sg/

Molly; and the egg-laying species: Dragon Fish, Goldfish, Cichlid, Angelfish, Gourami and Tetra.

Research and development

The aquaculture in Singapore is supported by a vibrant research and education ecosystem, including polytechnics, universities, private sector/research institutes and government organisations. These Institutes of Higher Learning (IHLs) and entities are crucial in the development of key competencies in the area of aquaculture genetics, nutrition, health and systems. Hence it is important to deepen our local research capabilities.

SFA's Marine Aquaculture Centre (MAC) is located on St John's Island in the open southern waters of Singapore and was established in 2003 by the former AVA to spearhead tropical aquaculture technology development for large scale hatcheries. Their R&D programmes focus on these areas:

Asian Sea bass

Since its setup, MAC has focused on genetics and broodstock development. Through its partnership with Temasek Life Sciences Laboratory (TLL) on Asian sea bass selective breeding programme, genetically superior Asian seabass lines that are able to produce seabass fry that grow at least 30 % faster than unselected seabass fry have been developed. The breeding programme with TLL utilises aquaculture genomic tools for more accurate selection of superior individuals without the use of genetic modification.

Large-scale fry production technology

MAC also undertakes R&D in large-scale hatchery production technology to ensure the long-term sustainable supply of marine fish fry. To date, MAC has successfully transformed hatchery production from an extensive outdoor pond system which requires large footprint and is vulnerable to weather changes to an intensive indoor closed-loop production system which allows large-scale production on a smaller footprint, enables the control of diseases and minimises waste discharges. This has led to 10x improvement in Asian sea bass fry survival and 100x intensification over the prevalent outdoor pond system. Hatchery protocols for other key marine fish species such as snapper, pompano and grouper have also been developed. Indoor hatchery production has since been adopted by our local hatcheries & nurseries.

Growing SG's aquaculture research ecosystem

MAC welcomes research institutes and IHLs to conduct collaborative R&D projects by contributing our expertise in the areas of fish husbandry such as breeding and live feed and hatchery production. MAC also provides shared facilities such as replicated tank systems for research and incubator space for test-bedding and commercialisation of R&D results. MAC also makes available key biological materials such as eggs, larvae, rotifers and microalgae for aquaculture research. These inputs of husbandry-related expertise, shared facilities and ease of access to biological materials will enable researchers in Singapore to conduct aquaculture R&D and to facilitate the translation.

Others

Besides R&D work, MAC also provides technical support to local farms to adopt sustainable farming practices and technology. Furthermore, MAC also hosts learning journeys for schools, conducts practical sessions and mentors interns from polytechnics and universities.

Country aquatic animal health infrastructure

The Singapore Food Agency (SFA) was formed on 1 April 2019, under the Ministry of the Environment and Water Resources (MEWR) to oversee food safety and security. Concurrently, all animal health-related services were transferred to the Animal & veterinary Service (AVS) of the National Parks Board. The SFA is the national authority responsible for all matters pertaining to food supply and safety in Singapore, including seafood production from aquaculture. The management of aquatic animal health cuts across several departments in the SFA.

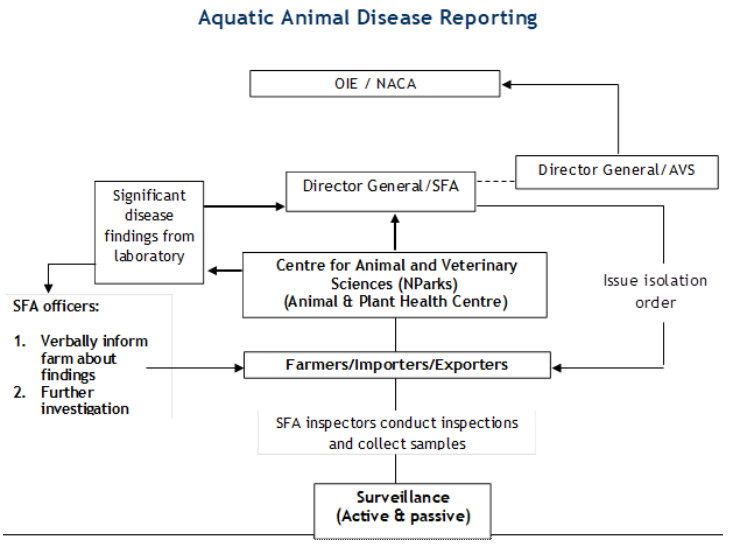
SFA inspection offices conduct regular inspections of the aquaculture farms, which consist of land and coastal farms. Inspections are conducted to check for compliance with licensing conditions, respond to alerts and to take enforcement action for non-compliances. The Veterinary Public Health Department (VPHD) coordinates the Disease Investigation Team (consisting of veterinarians and specialist inspectors), which is first-responder for investigation and clinical diagnosis of animal diseases detected at the aquaculture farms.

Samples are also routinely collected as part of surveillance programmes – market-size food fish, feed, oyster, biotoxin (for Harmful Algal Bloom), imported food fish fingerlings. The National Centre for Food Safety (NCFS) of SFA and Centre for Animal and Veterinary Services (CAVS) under National Parks Board (NParks) provides support via their diagnostic and testing capabilities in the areas of food safety and animal health, respectively. Aquaculture extension services are provided by extension staff under the Urban Food Solutions Division.

Disease monitoring reporting system

The national list of notifiable aquatic animal diseases relevant to aquaculture animals are Epizootic Ulcerative Syndrome (EUS), Koi herpes virus (KHV), Red Seabream Iridovirus (RSIV), Spring Viraemia of Carp Virus (SVCV) and White spot syndrome virus (WSSV). It is a legal requirement to notify AVS (NParks) if a notifiable or significant disease is suspected. Disease cards and posters are distributed to exporters and farmers for reference to increase their awareness and recognition of notifiable aquatic animal diseases.

For reports of fish kills or suspected or confirmed disease cases, the SFA will activate the Contingency Plan & Eradication Programme, which would include carrying out site investigation and taking samples for laboratory testing, before determining a suitable course of action based on the findings. Quarantine and suspension of movement may be imposed on the premise or area until



confirmation of disease following which total eradication of affected fish stock and disinfection of premises concerned will be carried out.

Commonly reported diseases/ impact of diseases on production

SFA and NParks implement national surveillance programs for significant viral diseases of food and ornamental fish respectively. Based on the information derived from these surveillance programs, government veterinarians, surveillance staff and extension personnel work closely with the aquaculture industry to control and manage aquatic diseases in Singapore.

Susceptible species are routinely collected for the following diseases:

a. Epizootic Ulcerative Syndrome (EUS)

b. Koi herpesvirus (KHV)

c. Spring Viraemia of Carp (SVC)

d. *Aeromonas salmonicida*

e. Iridoviruses (ISKNV & RSIV)

f. White spot syndrome virus (WSSV) for crustaceans.

In addition, samples will be taken for disease diagnosis should there be any diseased fish observed during surveillance of the aquaculture premises.

For OIE-notifiable diseases (i.e. KHV, WSSV), premises will be imposed with an isolation order, with compulsory culling of the affected batch and further investigation to ensure that other batches of aquatic animals in the premise are free from infection. In the case of non-OIE notifiable diseases, there is no regulatory requirement of compulsory culling. However, in most cases, operators will choose to voluntarily cull the batch to minimise disease spread.

Viral, bacterial or parasitic infections are common production diseases detected in marine fish in Singapore. These include Benedeniosis, Big-Belly (BB) Disease Syndrome, infections with *Streptococcus iniae* and *Tenacibaculum maritimum*,

Table 2. Control mechanisms for major pathogens

Major pathogens	Control measures
Finfish	
Megalocytivirus – Infectious Spleen and Kidney Necrosis Virus (ISKNV)	Stock vaccinated fish only. Recommend to cull diseased and potentially infected fish. Activation of heat shock proteins as part of disease management.
Megalocytivirus – Red Seabream Iridovirus (RSIV)	Stock vaccinated fish only. Cull diseased fish and vaccinate clinically healthy fish.
Singapore Grouper Iridovirus	Exclusion of virus via screening of incoming stock. Recommend to cull diseased and potentially infected fish.
Viral Nervous Necrosis Virus (VNNV)	Stock vaccinated fish or exclusion of virus via screening of incoming stock.
Koi Herpesvirus (KHV)	Cull diseased and infected fish.
Scale Drop Disease Virus	Exclusion of virus via screening of incoming stock.
Lates calcarifer Herpesvirus	Exclusion of virus via screening of incoming stock.
Big Belly Bacterial Disease	Pathogen exclusion. Raise early life stages of susceptible fish species, in low salinity RAS. Antibiotic treatment ineffective over several batches.
Streptococcosis	Stock vaccinated fish only, or Treatment with a suitable oral, in-feed antibiotic. Monitor for development of antibiotic resistance.
Vibriosis	Stock vaccinated fish only, or Treatment with a suitable oral, in-feed antibiotic. Monitor for development of antibiotic resistance.
Crustacean	
White Spot Syndrome Virus (WSSV)	Stock clean, disease-free crustaceans. Production in closed systems with high biosecurity. In event of detection, cull all diseased and infected shrimp.

Norcardiosis, Viral Nervous Necrosis (VNN), and Infectious spleen and kidney Necrosis virus (ISKNV).

Observation of clinical signs and gross external pathology of disease ornamental and food fin fish by the extension officers and inspectors are used as the first line in the detection of diseased fish. With a clinically normal population of fish, 30 pieces of fish are sampled each time per batch in order to achieve 95 % confidence in detection, assuming 10 % prevalence of the pathogen. When investigating a disease outbreak in a population of fish, minimum of 5–10 pieces of fish are collected from both moribund/affected and clinically normal fish.

R&D capability/initiatives

Singapore is conducting a carrying-capacity survey for its farm sites, with the aim of stipulating a production limit for farms so that they do not exceed the carrying capacity of a particular geographical area. This is intended to reduce adverse effects on water quality and the ecosystem.

The hydrodynamics of Singapore's waters are also being studied to identify hydrodynamically connected farm sites, to facilitate designation of farming areas and planning disease response.

Training needs

Training of Aquatic Animal Health Professionals for the aquaculture farms

SFA recognises the need for Aquatic Animal Health Professionals (AAHP) to develop, implement and review the biosecurity plans for the aquaculture farms. AAHPs are required to have aquaculture qualifications (eg. aquaculturists, aquatic veterinarians) and undergo further training in aquaculture farm biosecurity. SFA is working with institutions of learning to implement capability-building programmes to develop a pool of AAHPs in Singapore.

References

SFA website: http://www.sfa.gov.sg/

SFA Annual report 2017/2018: http://www.sfa.gov.sg/docs/default-source/publication/annual-report/ava-ar-2017-18.pdf

Yearbook of Statistics Singapore 2018: https://www.singstat.gov.sg/-/media/files/publications/reference/yearbook_2018/yos2018.pdf

https://www.sfa.gov.sg/food-farming/aqauculture-services/marine-aquaculture-centre

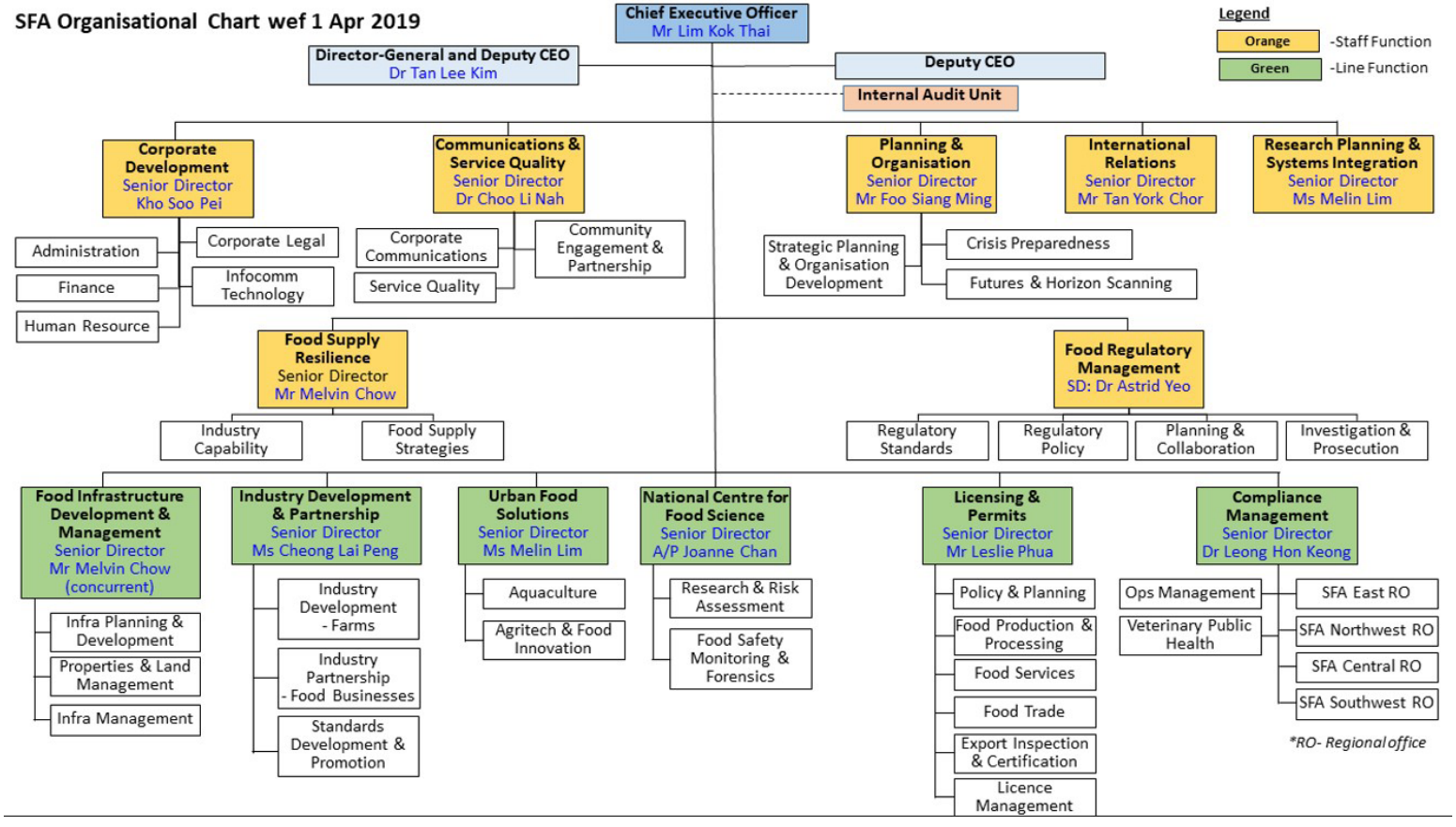


Figure 1. The Singapore Food Agency organizational chart

Report of Aquatic Animal Diseases in Thailand During January – June 2019

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Abstract

Aquaculture is an important industry in Thailand which has been established more than two decades ago. The cultured species are divided into two main groups; shrimp and finfish farming. The major cultured shrimp species are *Penaeus vannamei* (Pacific white shrimp), *P. monodon* (black tiger shrimp) and *Macrobrachium rosenbergii* (giant freshwater prawn), whereas the finfish are *Oreochromis* sp., *Lates calcarifer* and *Epinephelus* sp. Also, ornamental fish such as *Cyprinus carpio* (Koi carp), *Carassius* spp. (goldfish), and *Betta splendens* (fighting fish or betta). Disease outbreaks are the key factor that affect Thailand's aquaculture production and resulting in economic losses. The infectious diseases in aquaculture are mainly caused by viral and bacterial pathogens. In 2018, the reported shrimp pathogens are white spot syndrome virus (WSSV), yellow head virus (YHV) genotype 1, Taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (VP_{AHPND}), and microsporidian *Enterocytozoon hepatopenaei* (EHP). On the other hand, the reported pathogens in finfish are Betanodavirus causing viral nervous necrosis (VNN), Tilapia lake virus (TiLV) and *Streptococcus* sp. etc. In Thailand, the Department of Fisheries (DOF) is the competent authority for various aspects of aquatic animals including aquatic animal health.

Strategies to prevent and control diseases in aquatic animals include issuance of legislations/regulations, implementation of biosecurity measures, disease surveillance programs, capacity building, cooperation with international and national organizations.

Moreover, DOF has developed contingency plan in dealing with aquatic animal disease emergencies through the provincial fisheries officer. The provincial fisheries officer acts as director of emergency aquatic animal disease control center in each province, while Aquatic Animal Health Research and Development Division (AAHRDD) and Songkhla Aquatic Animal Health Research and Development Center (SAAHRC) serve as disease diagnosis and laboratory testing centers. Because of the above actions, we are capable of preventing and controlling disease outbreaks in the country. But during the occurrence of some diseases, we have no treatment to support and completely solve the problem. Example are viral diseases, unlike bacterial diseases which can be treated by using chemical or drug. Furthermore, there are a few researches that could be applied in farm level. Especially shrimp which has no adaptive immunity, so it is difficult to develop vaccine compare to fish. Therefore, DOF mostly recommended farmers to follow the good management practices on aquatic animal health for promoting sustainable aquaculture.

Introduction

Aquaculture is an important industry in Thailand and has been established more than two decades. The cultured species are divided into two main groups, shrimp and finfish farming. The major cultured shrimp species in Thailand are *Penaeus vannamei* (Pacific white shrimp), *P. monodon* (black tiger shrimp) and *Macrobrachium rosenbergii* (giant freshwater prawn), whereas the finfish species are *Oreochromis* sp., *Lates calcarifer* and *Epinephelus* sp. Also, ornamental fish such as *Cyprinus carpio* (Koi carp), *Carassius* spp. (goldfish), and *Betta splendens* (fighting fish or betta).

To raise the aquatic animal production to meet the global demand, the aquaculture industry has dramatically developed. However, development without good good aquaculture practices (GAqP) could be a negative factor that may reduce the ability of immune system or increase the susceptibility to diseases. Example is the higher stocking density in shrimp farms that led to the infection with yellowhead virus (YHV) in 1992 and white spot syndrome virus (WSSV) in 1994 (Briggs *et al*., 2004).

The infectious diseases in aquaculture are mainly caused by viral and bacterial pathogens. In 2018, the reported shrimp pathogens in Thailand are white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (Vp_{AHPND}), and microsporidian *Enterocytozoon hepatopenaei* (EHP). On the other hand, the reported pathogens in finfish are *Betanodavirus* causing viral encephalopathy and retinopathy (VER) also known as viral nervous necrosis (VNN), Tilapia lake virus (TiLV) and *Streptococcus* sp. etc. Aquatic animal diseases are the key factor that affect Thailand's aquaculture production and resulting in economic losses. Therefore, it is important to prevent and control the occurring and emerging diseases in aquatic animal.

In Thailand, aquaculture and fisheries are under the responsibility of the Department of Fisheries (DoF). The strategies for controlling aquatic animal diseases (Polchana, 2019) are:

1. Development of related legislations and regulations under the Animal Epidemic Act B.E. 2558 (2015),

2. Disease prevention and control system following the concept of biosecurity,

3. Disease surveillance programs,

4. Training program and seminar for DOF officers and farmers about diagnostic methods, disease knowledge etc.,

5. Cooperation with international and national organizations. Moreover, DOF has developed contingency plan for dealing with aquatic animal disease emergencies by the provincial fisheries officer in the responsible area.

Shrimp diseases

Country update on diseases affecting Shrimp

Epidemiology

Prevalence of the disease

Severity and economic impact**.** During January to June 2019, the highest prevalence of shrimp disease was EHP,

(Table 1) which has been found in shrimp farms, including hatcheries, nurseries and grow-out farms. It causes growth retardation and increased size variability instead of mortality. On the other hand, EHP may be a cause of white feces syndrome (WFS).

MrNV was the second in disease prevalence but there was no report on its impact that affect the production. Moreover, the most of MrNV-positive animals did not show any clinical sign.

Species affected

- WSSV: Black tiger prawn (*Penaeus monodon*), Pacific white shrimp (*Penaeus vannamei*) and crayfish
- TSV: *L. vannamei*, *Macrobrachium lanchesteri* and some crab species
- YHV, EHP, and Vp_{AHPND} : *P. monodon* and *P. vannamei*
- *Mr*NV : *M. rosenbergii* and *M. lanchesteri*

Stages affected. Juvenile stage and small shrimp.

Risk factors associated with the different shrimp diseases are presented in Table 2.

History of occurrence

YHV, WSSV, *Mr*NV and TSV were found in Thailand more than 15 years ago and became endemic disease (Ganjoor, 2015). Presently, only WSSV has been reported as the cause of disease outbreak in specific area, whereas no report for others. Vp_{AHPND} occurred since 2012 and causing the big economic losses on shrimp farming during 2014-2015. In 2004, EHP was reported as new shrimp pathogen in *P. monodon* and the prevalence increase after Vp_{AHPND} outbreak (Putth and Polchana, 2016).

Table 1. Prevalence of shrimp diseases in Thailand (January–June 2019)

Diseases pathogens	Prevalence (total specimen)
1. Taura syndrome virus (TSV)	0.71 % (4,514)
2. White spot syndrome virus (WSSV)	1.20 % (5,258)
3. Yellow head virus (YHV)	1.36 % (1,760)
4. Infectious hypodermal and hematopoietic necrosis virus (IHHNV)	1.03 % (4,359)
5. Enterocytozoon hepatopenaei (EHP)	22.38 % (3,602)
6. Acute hepatopancreatic necrosis disease caused by *Vibrio parahaemolyticus* (Vp_{AHPND})	2.46 % (4,806)
7. *Macrobrachium rosenbergii* nodavirus (MrNV)	10.14 % (148)

Table 2. Risk factors of shrimp diseases

Diseases pathogens	Risk factors
1. TSV	salinity, vertical/horizontal transmission
2. WSSV	season change, water temperature, vertical/horizontal transmission
3. YHV	water quality, vertical/horizontal transmission
4. IHHNV	vertical/horizontal transmission
5. EHP	high organic matter, horizontal transmission
6. Vp_{AHPND}	high organic matter, temperature, horizontal transmission, salinity
7. MrNV	vertical/horizontal transmission

Diagnosis

Disease signs

Diagnostic methods employed. Observing the gross signs and detection by molecular technique using real-time PCR or conventional PCR.

Table 3. Clinical signs of shrimp diseases

Disease pathogens	Clinical signs
1. TSV	tail fan and pleopods distinctly red, multifocal melanized lesions on the cuticle
2. WSSV	white spots embedded within the exoskeleton, decreased or absent feed consumption and abnormal swimming behavior
3. YHV	yellow hepatopancreas, mass mortality in early to late juvenile stages
4. IHHNV	a deformed rostrum bent to the left or right, a marked reduction in food consumption
5. EHP	growth retardation, soft shells, lethargy, reduced feed intake and empty midgut
6. Vp_{AHPND}	pale-to-white hepatopancreas, empty gut
7. MrNV	whitish discoloration in the abdominal segment

Prevention and control

Approaches (chemical, biological): successful and failed approaches

- GAqP (i.e. pond and water preparations, water quality, stock density, feed control and quality)
- Probiotic application in farm

Fish diseases

Country update on diseases affecting Fish (FW/BW/MW)

Epidemiology

Prevalence of the disease

Species affected

- VER: Asian Sea bass (*Lates calcarifer*)
- TiLV: Tilapia (*Oreochromis* spp.)

Stages affected. VER and TiLV were mostly detected in young fish.

Table 4. Prevalence of fish diseases in Thailand (January–June 2019)

Disease pathogens	Prevalence (total specimen)
1. Viral encephalopathy and retinopathy (VER)	4.62 % (520)
2. Tilapia lake virus (TiLV)	17.65 % (221)

Risk factors

History of occurrence

The TiLV was found in Thailand during 2015-2016.

Diagnosis

Disease signs

VER: darkened skin, deformation of the backbone, abdominal distension, skin lesions, and fin erosion (Toffan, 2017)

TiLV: abnormal swimming, severe anemia, bilateral exophthalmia, skin erosion and congestion, scale protrusion, and abdominal swelling (Jansen & Mohan, 2017)

Diagnostic methods employed

Cell culture and molecular techniques

Table 5. Risk factor of fish diseases

Disease pathogens	Prevalence (total specimen)
1. VER	water temperature, salinity, ammonia level, vertical/horizontal transmission
2. TiLV	water temperature, salinity, vertical/horizontal transmission

Prevention and control

Chemical and biological approaches

Disease screening in broodstock and monitoring in fish/shrimp farms.

Scientific research done

Scientific studies conducted/ongoing

Country implementation of Aquatic Emergency Preparedness and Response Systems for effective management of aquatic animals

Monitoring system/mechanism on emerging/ existing transboundary diseases (especially the OIE-listed) in the region

Thailand has developed the Import and export regulations.

Entry level (importation)

- Requirement of health certificate from origin

  - Marine shrimp: Free of WSSV, IHHNV, YHV, TSV, IMNV, Vp_{AHPND}, EHP, SHIV

- Quarantine process and disease diagnoses at arrival

- Disease surveillance for targeted fresh/frozen products such as marine shrimp and asian sea bass

Exit level (exportation)

Regulation on registration of aquaculture establishment for exportation of aquatic animals. Under this regulation, the aquaculture establishment need to be registered and monitored the targeted diseases, which is based on the OIE-listed diseases.

Personnel competencies on recognition/ diagnostic capability / capacity and reporting of a disease emergency

There are several organizations of DoF involved in aquatic animal health, such as Aquatic Animal Health Research and Development Division, Inland Aquaculture Research and Development Division, Coastal Aquaculture Research and Development Division and Fisheries Provincial offices. The related staffs (i.e. Fisheries Biologists, Fisheries Provincial officers) have been trained for primary diagnosis of diseases and some staffs are the specialists in aquatic animal diseases.

For diagnostic laboratory, there are twenty laboratories which belonging to DoF, including two national reference laboratories and nineteen regional laboratories. These laboratories are located in different regions around the country. On the other hand, there are private and university laboratories that are capable of aquatic animal disease diagnosis. Moreover, DoF has developed contingency plan for dealing with aquatic animal disease emergencies by the provincial fisheries officer in the responsible area. The provincial fisheries officer acts as director of emergency aquatic animal disease control center in each province, while Aquatic Animal Health Research and Development Division (AAHRDD) and Songkhla Aquatic Animal Health Research and Development Center (SAAHRC) act for disease diagnosis and laboratory testing.

In addition, DoF staff are responsible to report the disease emergency in their area.

Also, fish farmers regularly report disease outbreak to the DoF staff.

Surveillance systems

There are two surveillance programs: passive and active. Passive surveillance programs are for targeted and non-targeted diseases; while active surveillance programs are for targeted diseases only. The disease surveillance system is an action plan of DoF to control aquatic animal diseases in the country. The surveillance system consists of active and passive surveillance programs.

Active surveillance program

- To declare country free status : IMNV, CMNV, NHPB etc.
- National surveillance to obtain disease status: WSSV, IHHNV, YHV, TSV, IMNV, VpAHPND, EHP, NHPB, KHV, SVCV, TiLV etc.
- Diseases farms should be from:

  -Exportation farm list : WSSV, IHHNV, YHV, TSV, IMNV, Vp_{AHPND}, EHP, MrNV/XSV, crayfish plague, TiLV, VNN, KHV, SVCV etc.

  -Hatchery farms of *P. vannamei*: WSSV, IHHNV, YHV, TSV, IMNV, Vp_{AHPND}, EHP
- Listed *P. monodon* hatchery and grow-out farms in cluster : WSSV, IHHNV, VpAHPND, EHP, YHV

Passive surveillance program

For targeted and non-targeted diseases. The samples are the aquatic animals which are suspected of having disease.

References

Briggs, M., Funge-Smith, S., Subasinghe, R., & Phillips, M. 2004. Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. 40.

Ganjoor, M. 2015. A Short Review on Infectious Viruses in Cultural Shrimps (Penaeidae Family). Fisheries and Aquaculture Journal, 06. https://doi.org/10.4172/2150-3508.1000136

Jansen, M. D., & Mohan, C. V. 2017. Tilapia lake virus (TiLV): Literature review. 12.

Polchana, J. 2019. Aquatic emergency preparedness and response system in Thailand. 51–55. https://repository.seafdec.org.ph/handle/10862/3463

Putth, S., & Polchana, J. 2016. Current status and impact of early mortality syndrome (EMS)/acute hepatopancreatic necrosis disease (AHPND) and hepatopancreatic microsporidiosis (HPM) outbreaks on Thailand s shrimp farming. 79–87. https://repository.seafdec.org.ph/handle/10862/3094

Surachetpong, W., Janetanakit, T., Nonthabenjawan, N., Tattiyapong, P., Sirikanchana, K., & Amonsin, A. 2017. Outbreaks of Tilapia Lake Virus Infection, Thailand, 2015–2016. Emerging Infectious Diseases, 23(6), 1031–1033. https://doi.org/10.3201/eid2306.161278

Toffan, A. 2017. Viral encephalopathy and retinopathy. In P. T. K. Woo & R. C. Cipriano (Eds.), Fish viruses and bacteria: Pathobiology and protection (pp. 128–146). CABI. https://doi.org/10.1079/9781780647784.0128

Department of Animal Health (DAH) Report of Viet Nam Emergency Diseases - Prevention and Control Shrimp Diseases in Viet Nam From 2016 to 2018

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Abstract

Acute Hepatopancreatic Necrosis Disease (AHPND), known as Early Mortality Syndrome (EMS) before 2013, was first reported in Vietnam from the Mekong River Delta without laboratory confirmation in the end of 2010. *Vibrio parahaemolyticus* was recognized as the causative agent of AHPND reported by Dr. Tran Loc in 2013. From 2010 to 2015, AHPND spread quickly to a wide range of shrimp production areas in Viet Nam. White Spot Disease (WSD), in dual infection with AHPND, cause serious disease, each year nearly 1
% in total culture area stopped operation. Although the diseases decreased significantly from 2013 to 2018, AHPND and WSD are still the most serious problem encountered by shrimp farmers in main shrimp production areas. Both tiger shrimp (*Penaeus monodon*) and whiteleg shrimp (*Litopenaeus vannamei*) have been infected with AHPND and WSD; most cases occur 15–60 days after stocking. AHPND and WSD occur year round; however, it is most frequently observed between March to September. Vietnam has alleviated the effect of AHPND and WSD effectively in the current year. This can be attributed to the application of multiple solutions to prevent and control AHPND and WSD such as (1) improve farmers' production conditions, awareness, facilities and bio-security measures; (2) implement active surveillance in main shrimp production areas for early warning; (3) broodstock and postlarvae are not accepted if positive for any OIE listed diseases; (4) movement control to prevent spreading disease; (5) encourage developing disease-free farms; (6) improve capacities and complete aquatic animal health system.

Introduction

Viet Nam has 28 coastal provinces with more than 3,260 km coastal line with large area of beach and many river systems such as Red river system and Mekong river system. Those are not only appropriate environment and condition for developing agriculture including brackish shrimp and catfish but also to exploit aquatic resources. With this advantage, the aquaculture industry of Vietnam develops with high speed, especially shrimp and catfish, and become one of the most aquatic product exporters in the world that contributes so much in GDP of Vietnam. From 2016 to 2018, the total aquatic animal product was 6.7 million tones, 7.3 million tonnes and 7.7 million tones, respectively. Viet Nam exported aquatic animal products and obtained about USD 7.1 billion, 8.3 billion and 9 billion from 2016 to 2019, respectively.

Viet Nam has approximately more than 743,000 hectares (ha) of brackishwater shrimp farms. The main area stocked with brackishwater shrimp in Viet Nam was the Mekong River Delta (southern Viet Nam) with about 565,000 (ha) (92 % in total shrimp cultivate surface of Viet Nam). Mekong River Delta contributes more than 95 % shrimp product of Viet Nam. In this area, all coastal provinces develop shrimp farms such as Ben Tre, Tien Giang, Tra Vinh, Soc Trang, Bac Lieu, Kien Giang and Ca Mau provinces. Based on the management systems, farm facilities,infrastructures, and shrimp stocking density, the shrimp farms are divided into 6 types: high technical – intensive, intensive, semi-intensive, extensive, improved extensive and integrated rice and shrimp farming. The shrimp farms are often impacted by diseases such as WSD, AHPND (or EMS), white faeces disease and other diseases. Among these diseases, WSD and AHPND effected shrimp production in Viet Nam the most. EMS was first reported in Viet Nam in So Trang province in the end of 2010. In 2011, 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 provinces. It made shrimp production reduced sharply. The Ministry of Rural Development (MARD) tried their best to identify the pathogen and carried out prevention and control activities. MARD also called for help from international organizations such as, OIE and FAO. *Vibrio parahaemolyticus* with phage was identified as the causative agent of AHPND (Lightner *et al*., 2013). WSD occurred in shrimp production areas from 1994–1995 and have been reported in many production areas in Viet Nam (from the North to the South). In 2015, 5,369 ha were affected by AHPND in 23 provinces compared with 23,850 ha in 2014 (Hien, 2016). In recent years, AHPND and WSD often occur during the main shrimp production period, but Viet Nam has applied various interventions to effectively control these diseases.

Shrimp diseases

In 2018, Viet Nam lost 1.6 % in total culture area because of diseases. Shrimp farms were affected by AHPND, WSD, MBV, and other diseases. Among these, AHPND and WSD are the most serious thus, the Vietnamese Government focused on the prevention and control of these diseases. Diseases usually occur in small farms where farmers did not apply or follow biosecurity practices. From 2008 to 2019, Taura Syndrome Virus (TSV), Yellow-head Virus (YHV) had not been detected in shrimp farms, although many active surveillance programs were conducted by local government and center government.

Status of AHPND

Epidemiology

Severity and economic impact

From 2016 to 2018, less than 1 % of the total culture area was affected by AHPND (**Table 1**). Lowest was observed in 2018, wherein only 0.76 % of the total culture area was affected compared to in 2016 (0.94 %) and 2017 (96 %).

AHPND caused serious economic losses in shrimp production in Viet Nam. It was estimated that annually, a total of more than USD 50 million was estimated to be lost due to this disease annually. However, Viet Nam still needs to carry out cost-effective analysis to identify the exact economic figures.

Species affected

Both tiger (*Penaeus monodon*) and white-leg shrimp (*Litopenaeus vannamei*) have been infected with AHPND. **Table 1** shows that percentage of total culture area affected by AHPND were different between tiger shrimp and white-leg shrimp. White-leg shrimp was more susceptible to AHPND than tiger shrimp (more than 9 times). The tiger shrimp being a local species is adapted better to the environment in Viet Nam compared with the imported white-leg shrimp. Additionally, the white-leg shrimp was usually cultured at higher density than tiger shrimp. More epidemiological research studies to identify species factor that may be highly associated with risk of AHPND infection.

Stages of the shrimp affected

On average, shrimps were infected with AHPND 35–40 days after stocking, a wide range from 0 up to 149 days, but a narrower period is about from 16 to 60 days after stocking (**Table 2**).

Risk factors

Risk factors identified during the 2011 to 2018 surveillance program are the following: large culture area of farm, sun-dried sediment of culture pond method, located closely to other farms that used the same AHPND-affected water source, the depth of pond (more than 1.2 meter or less), pond size (bigger 0.25 hectares and less) and presence of wild animals.

History of occurrence

In 2010, unconfirmed AHPND outbreaks were first reported in the Mekong River Delta (main shrimp production region). In 2011, the disease spread to a wide range of shrimp production areas, including Ninh Thu□n (16 ha), Soc Trang (1,719 ha), Bac Lieu (346 ha) and Ca Mau (3,493 ha) provinces (OIE, 2012). In 2012, AHPND continuously spread to a wide range of shrimp production areas, not only along the Mekong Delta River such as Soc Trang (2,100 ha), Tra Vinh (1,642 ha), Bac Liêu

Table 1. AHPND impact on shrimp industry

	Year		
	2016	**2017**	**2018**
Proportion of disease(%)	0.94	0.96	0.77
Tiger Shirmp (%)	0.44	0.45	0.35
White-leg Shirmp (%)	4.53	4.12	3.36

(2,000 ha), Ca Mau (4,007 ha), Ben Tre (133 ha) but also in middle of Viet Nam (Quang Ngai, Binh Dinh, Ninh Thuan, Phu Yen, Khanh Hoa) (OIE, 2013)

The project TCP/VIE/3304 was conducted by FAO *"Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam"* to help Viet Nam. One of the output of the project is the identification of *Vibrio parahaemolyticus* as the causative agent of AHPND (Tran Loc *et al*., 2013).

Currently, AHPND is continuously causing outbreaks in shrimp production areas in Viet Nam and the temporal pattern of the disease is presented in **Figure 1.**

Table 2. Stages of the shrimp affected AHPND

Age (after stonking)	Year 2016 Cum(%)	Year 2017 Cum(%)	Year 2018 Cum(%)
01-15	1.73	0.76	1.57
16-30	24.69	29.82	25.15
31-45	50.58	48.16	49.36
46-60	16.87	15.97	15.22
61-75	4.85	3.82	5.10
76-90	1.02	1.38	2.73
91-105	0.21	0.08	0.36
106-120	0.05	-	0.15
121-135	-	-	0.22
136-150	-	-	0.15

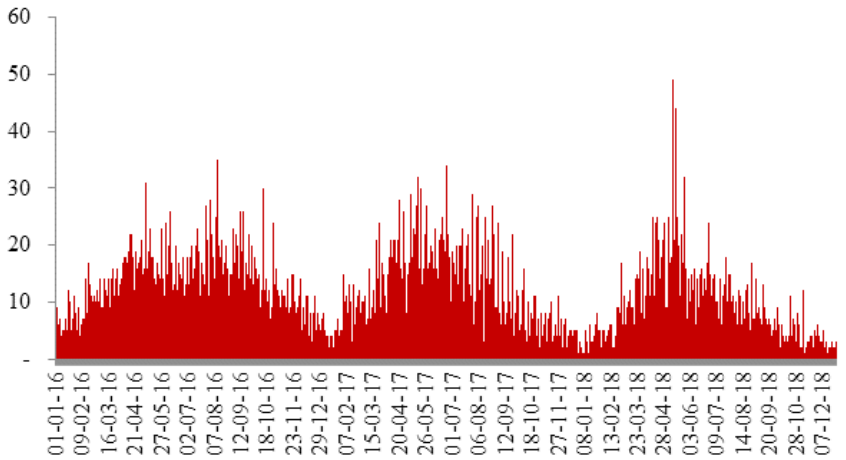

Figure 1. The epidemiological curve showing the temporal distribution of AHPND outbreaks occurred in Viet Nam from 2016 to 2018

Diagnostic methods employed:

Disease signs

Infected shrimps have the following clinical signs: weakness, empty or little food in gut and pale (**Figure 2**), soft shell, atrophied (**Figure 3**) and hard to crush or swollen and easier to broken hepatopancreas; and shrimps die suddenly with high mortality rate in ponds. **Figure 4** shows healthy shrimp.

Diagnostic methods employed:

Real time – PCR was used to test AHPND. The test protocol followed the Standard/ Criteria no.: TCCS 01:2016/TY-TS of DAH,

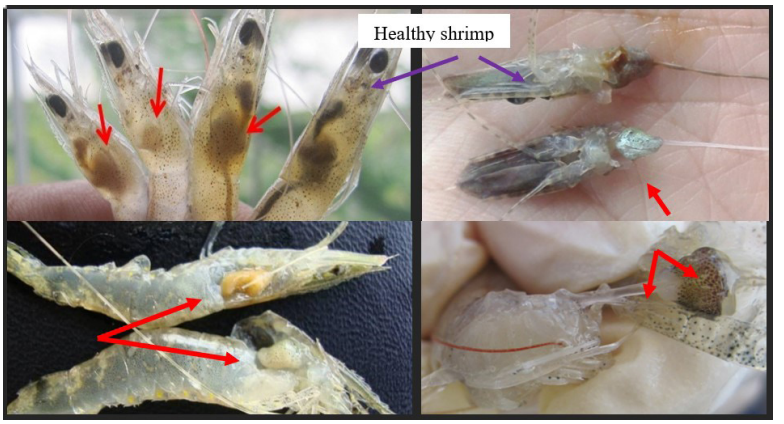

Figure 2. Clinical signs of AHPND (red arrow) in shrimp: empty or little food in gut, decreased color of cover (pale) and pale hepatopancreas (Sources: Dr. Lighner and TCP/VIE/3304 (E))

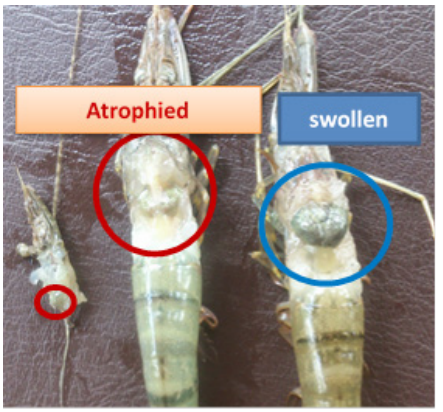

Figure 3. Clinical sigh of shrimps infected with AHPND: swollen (encircled blue) and atrophied (encircled red) hepatopancreas

Figure 4. Healthy *P. monodon*

and used by all laboratories of DAH. This method uses primer set 3 (AP3): VpPirA-F, VpPirA-R and probe, kit Platinum qPCR SuperMix-UDG

PCR also was used to test AHPND. The test protocol followed the Standard/ Criteria no.: TCCS 02: 2014/TY-TS of DAH, and used by all laboratories of DAH. This method uses primer set 3 (AP3): VpPirA-F, VpPirA-R, Taq PCR Master Mix kit.

Status of WSD in shrimp

Epidemiology

Severity and economic impact

In 2018, WSD was detected in 0.82 % of total of cultivate area. The proportion of infected AHPND area increased in comparison with 2016 and 2017. From 2016 to 2018, the proportion of infected area was less than 1 % (**Table 3**).

Table 3 indicates that proportion of infected WSD were significantly different between tiger shrimp and white-leg shrimp. White-leg shrimp was more susceptible to WSD than tiger shrimp (more than 5.7 times) thought out a 3-year period. This is for the same reason mentioned for AHPND.

Stages of the shrimp affected

On average, shrimps were infected with WSD after stocking for 35 days (a wide range from 1 up to 195 days), but a narrower period is about from 16 to 60 days after stocking (**Table 4**).

WSD occur the whole year from January to December annually. However, outbreaks were recorded the most from March to September, the period considered best for shrimp culture in Viet Nam (**Figure 5**).

Diagnostic methods employed: PCR or RT-PCR or based on clinical signs.

Disease signs

Infected tiger shrimp or white leg shrimp has these symptoms observed at farm level: weakness, empty or little food in gut, swimming water surface, discoloration of the exoskeleton (pale) and at least one of specific signs: white spot (0.2–0.5 cm) in the cephalothorax and the uropod, that could not remove by temperature or brushing.

Diagnostic methods employed:

Real time – PCR was used to test WSD. The testing protocol followed the Standard/ Criteria no.: TCCS 01: 2014/TY-TS of DAH, apply to all Labs of DAH. This method uses primer: WSSV1011F, WSSV1079R and probe (WSSV-p), kit Platinum qPCR SuperMix-UDG.

Table 3. WSD impact on shrimp industry

	Year		
	2016	**2017**	**2018**
Proportion of disease (%)	0.57	0.72	0.82
Tiger shrimp (%)	0.34	0.42	0.50
Whiteleg shrimp (%)	2.28	2.59	2.84

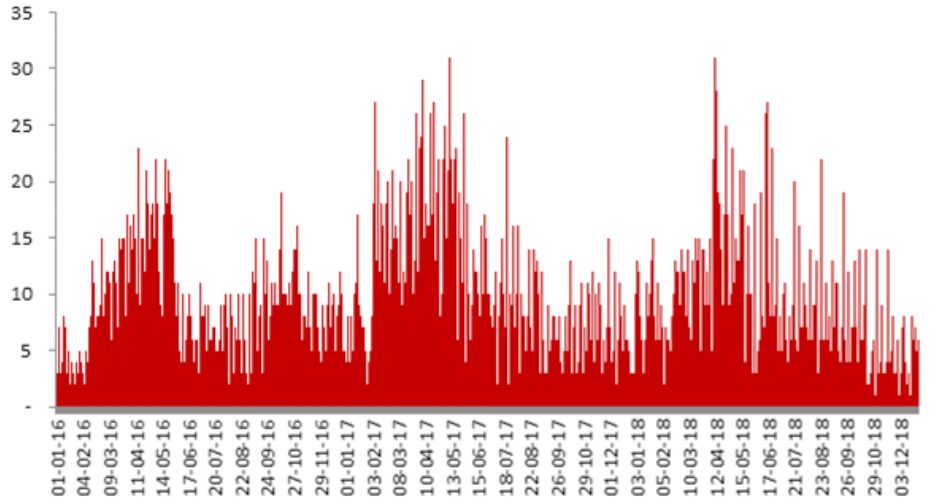

Figure 5. The epidemiological curve showing the temporal distribution of WSD outbreaks occurred in Viet Nam from 2016 to 2018

Table 4. Stages of shrimp affected with WSD.

Age (after stocking)	Year2016 Cum (%)	Year 2017 Cum (%)	Year 2018 Cum (%)
1-15	1.31	1.1	1.96
16-30	27.11	28.37	28.35
31-45	43.23	42.23	45.66
46-60	19.15	23.95	15.97
61-75	6.06	2.8	5.3
76-90	2.43	1.07	1.52
91-105	0.34	0.3	0.78
106-120	0.15	0.12	0.41
121-135	0.11	0	0
136-150	0.04	0.06	0
151-165	0	0	0.04
181-195	0.07	0	0

Figure 6. Clinical sigh of *P. monodon* infected with WSD: spots on the head and tail cover of infected shrimps (source: Dr. Bui Quang Te)

Prevention and control (AHPND, WSD and other aquatic animal diseases)

The following techniques and policies were developed and applied to prevent and control diseases in shrimps:

Techniques

Improve biosecurity measures at both hatcheries and shrimp production farms. New techniques and standards for cultivation and management, such VietGAP, GlobeGAP, PRC, RT-PCT, were introduced to all shrimp production areas. All DAH's laboratories (n = 7) are currently using the standardized PCR protocol to test for agent causing AHPND. In Addition, there are more than 22 provincial laboratories.

Improving the capacity of farmers and staff of the aquatic animal health services by conducting several training courses. The subject and content of the training courses are: epidemiological characteristics of disease, disease and diagnosis methods, good aquaculture practices, management and response to disease, technique for recording, managing and analysis of survey data and report.

Trainings are also offered to improve the capabilities of staff of private companies. The subject and content are:

(1) policies,

(2) sampling for test shrimp products before exporting,

(3) biosecurity,

(4) disease surveillance technique and design of surveillance program to prove a disease free farm status.

DAH sends high qualified technical staff teams to farmers to work with them and local veterinary agencies to carry out prevention and control activities. The DAH team also helped farmers and local veterinarians to identify disease and if detected to apply interventions. They also instruct local veterinary staff to improve their knowledge on the disease, epidemiological techniques for outbreak investigation, surveillance, data collection, cleaning and analysis.

Broodstock and postlarvae producers and shrimp farms are encouraged to build disease-free farms. At present, 4 corporate-owned companies conduct bio-security and active surveillance programs to prove disease-free facilities following the Australia Act (based on OIE code) or/ and Vietnam regulation.

Active surveillance program

Carry out active surveillance programs for AHPND and WSD which is funded by farmers, local government and central government. For example, in 2015, the central government carried out active surveillance in 8 main shrimp production provinces: Quang Ninh, Nam Dinh, Ha

Tinh, Ninh Thuan, Binh Thuan, Soc Trang and Ben Tre. From 2017–2020, local government and central government carried out the National Plan for disease surveillance in shrimps and pangasius in the period between 2017 and 2020 in main shrimp production areas including stock to assess the prevalence proportion of disease (AHPND, WSD, HYD, IHHN)

Improve passive surveillance system: The standardized reporting system from the farm level to national level was used and online disease reporting system was developed led by DAH and Sub-DAH.

Build disease-free establishment:

Support and encourage the development of animal disease-free establishments. One Corporation was accredited disease-free with WSSV, AHPND, YHV, TSV and EHP (meet OIE standard); and 3 others on the process to be proven disease-free.

Other actions

- Established the National Steering Committee for Prevention and Control of Disease in Brackish Shrimps in November 2014. One of the committee's responsibilities is to advise Ministers to develop and carry out disease interventions in shrimps. Implement and inspect the annual disease prevention and control program of each province.

- Enhance capacity of laboratory system by investing and applying new techniques for Provincial Veterinarian Offices (sub-DAH: sub-Department of Animal Health) and Region of Animal Health Offices, take part in inter-laboratory testing.

- MARD allowed the use of some drugs, chemicals and biotic products to effectively control disease; however, farmers must ensure that aquaculture products are safe for human consumption, free from drug or chemical residues. Farmers are advised to use the correct doses of the product and to observe withdrawal time to comply with regulations on residue and food safety.

- Research Institute for Aquaculture (RIA) No. 1, 2, 3 and universities research the disease, risk factors and new methods to treat and respond to AHPND which includes finding antibiotics effective against the disease and a list of carrier animals; and aquaculture practices that will mitigate the effect of AHPND.

Scientific research done: scientific studies conducted/ongoing

In 2016, surveillance program for WSSV, AHPND, IHHNV and EHP in brackish water farmed shrimp were carried out. In 2017 and 2020, the National Plan for disease surveillance in pangasius and shrimp production including stock to assess prevalence proportion of disease (AHPND, WSD, YHV, IHHNV) was implemented.

From 2016 to 2018 DAH instructed other provinces to carry out active surveillance and formulate provincial plan for the prevention and control of OIE listed notifiable aquatic animal diseases using their own local budget and resources.

Research gaps

Epidemiological studies about risk factors, cost-effective study to determine exact

economic figures, AMR study especially in local government are lacking

Surveillance designs donot satisfy the guidelines set by the DAH or OIE in almost main aquaculture area due to the limited budget and resources for aquatic animal health.

Evaluation of the efficiency of aquatic animal health services.

Pond level identification of diseased shrimp

Because infected shrimps usually die in very short time, small size of shrimps and many cases do not show clinical signs that it is quite difficult for farmers to identify the disease correctly.

Treatment

Extensive, improved extensive and integrated rice and shrimp farms have normally poor farmers. They have little knowledge and capacity about diseases, bio-security, and response when diseases occur in farm or be threatened by AHPND from neighboring farms.

During disease outbreaks, small farms usually drain water directly to rivers without water treatment. This is one of the transmission methods by which disease spread quickly and become unmanageable in large areas.

Reporting

Attitude and awareness of many farmers are low on reporting diseases to local staff in the commune (In Vietnam, each commune have at least 1 field staff to record and report animal disease).

Country implementation of Aquatic Emergency Preparedness and Response Systems for effective management of aquatic animal disease outbreaks

Policy and Act.

Viet Nam has several laws related to the management of aquatic animal diseases that both farmers and AAH staff should follow. Among these are the following:

- Decree number 35/2016/ND-CP dated 15 May 2016 stipulating detail of some articles of Animal Health Law.
- Circular number 04/2016/TT-BNNPTNT dated 10 May 2016 stipulating prevention and control of aquatic animal diseases.
- Circular 10/2016/TT-BNNPTNT dated 01 June 2016 stipulating list of drugs are used and banned for animal.
- Circular 13/2016/TT-BNNPTNT dated 02 June 2016 stipulating management of drugs for animal.
- Circular number 14/2016/TT-BNNPTNT dated 02 June 2016 stipulating animal disease-free zones and establishments
- Circular number 26/2016/TT-BNNPTNT dated 30 June 2016 stipulating quarantine of aquatic animals and aquatic animal products, it applies to import products.

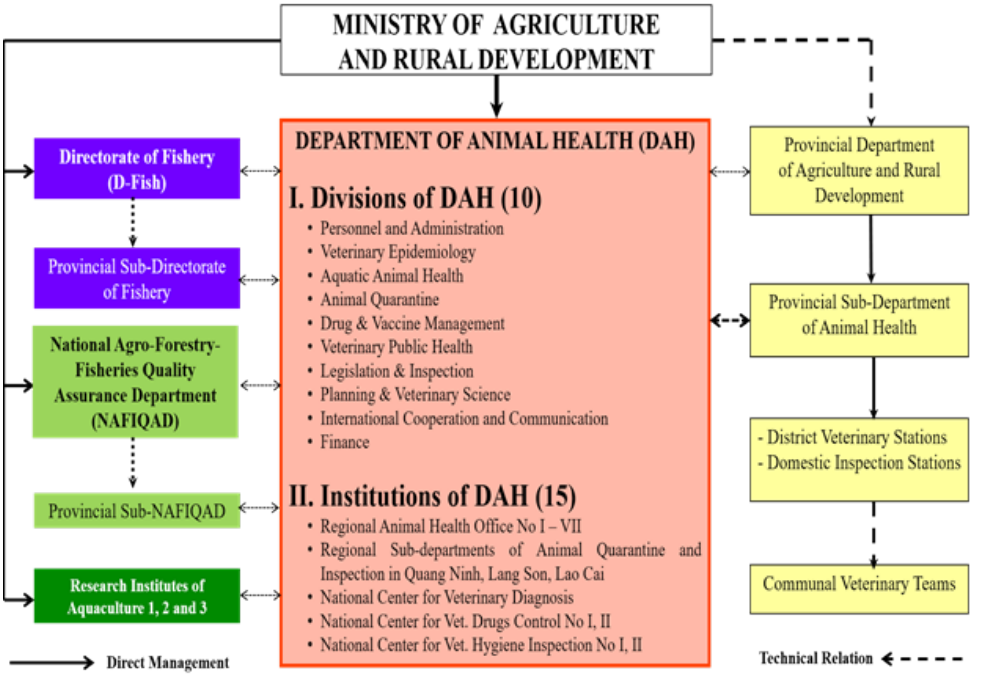

Figure 7. Organizational structure of animal health system of Viet Nam

- Circular number 36/2018/TT-BNNPTNT dated 25 Dec 2018 adjusts the Circular number 26/2016/TT-BNNPTNT.

Monitoring system

The Department of Animal Health has 10 functional divisions located in Hanoi, 7 Regional Animal Health Offices I-VII throw country, NCVD, NCVDC I and II, NCVHI No. I and II, 3 Regional sub Department of Animal Quarantine in Lang Son, Lao Cai and Quang Ninh (3 provinces of border gate) (**Figure 7**).

Viet Nam also have 63 Sub-Department of Animal Health (Sub-DAH) in 63 provinces. Each district has one District Veterinary Station and each commune has at least 01 field staff.

Personnel competencies on recognition/diagnostic capacity/ capacity and reporting of a disease emergency

Laboratory system

The aquatic animal health system has national laboratories, under DAH's management and local laboratories under provincial sub DAH and sub NAFIQAD. It has 41 public laboratories at both levels, of which 20 were granted with ISO 17025 and accredited by competent authority. The 41 public and 2 private laboratories are as follows:

- Central level: 8 aquatic animal disease testing laboratories of the Regional Animal Health Offices (RAHO) and the National Centre for Veterinary Diagnosis (NCVD).

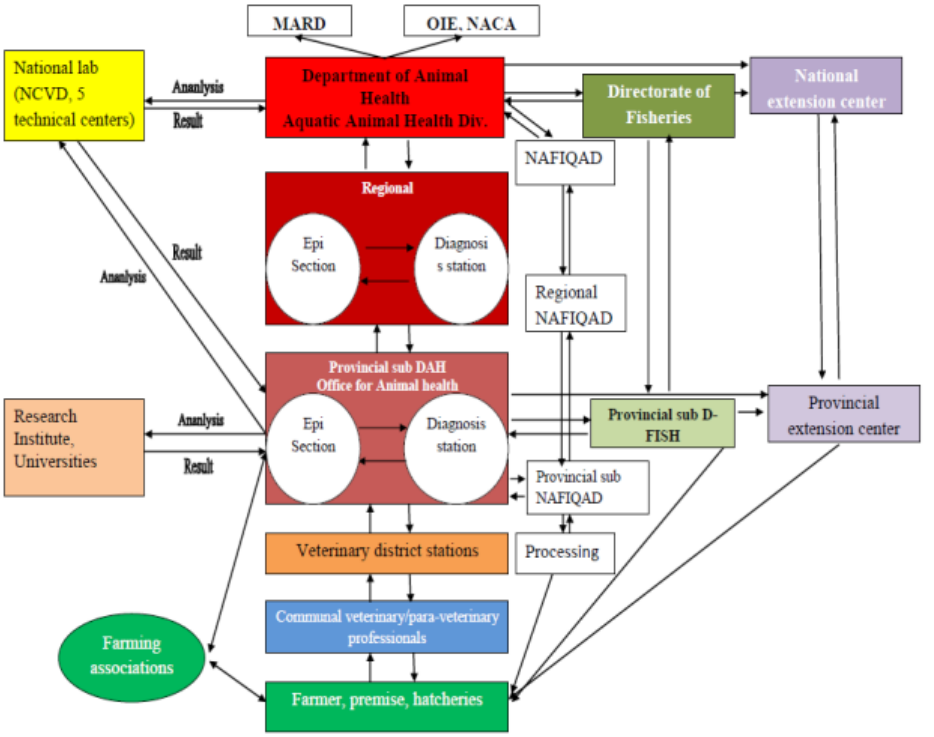

Figure 8. Gathering information and response disease system

- Local level: provincial sub DAH 27 laboratories (testing by conventional and Real-time PCR techniques).
- Agro-Forestry and Fishery Quality Assurance Department's management (NAFIQAD): 6 labs accredited in line with ISO 17025.
- and (2) Laboratories at three Research institutes for Aquaculture and fisheries universities.

Some private laboratories are also accredited to provide testing service for aquatic disease.

Aquatic animal quarantine system:

The agencies belonging to the Aquatic animal health system performing aquatic animal quarantine function are the Animal Quarantine section in DAH (DAH headquarter, 7 Regional Animal Health Offices I-VII), 3 Regional sub Department of Animal Quarantine in Lang Son, Lao Cai and Quang Ninh (3 provinces of border gates) and 63 provincial Sub-DAHs.

The DAH manages import and export of animals and animal products, including aquatic animals and issues Health certificate for imported/exported aquatic animals/products (except Health Certificate for aquatic animal products exported for human consumption which is granted by NAFIQAD), 63 Sub-DAHs manage local transportation of animals and animals products through animal Quarantine Checking Points along transportation roads (**Figure 9**).

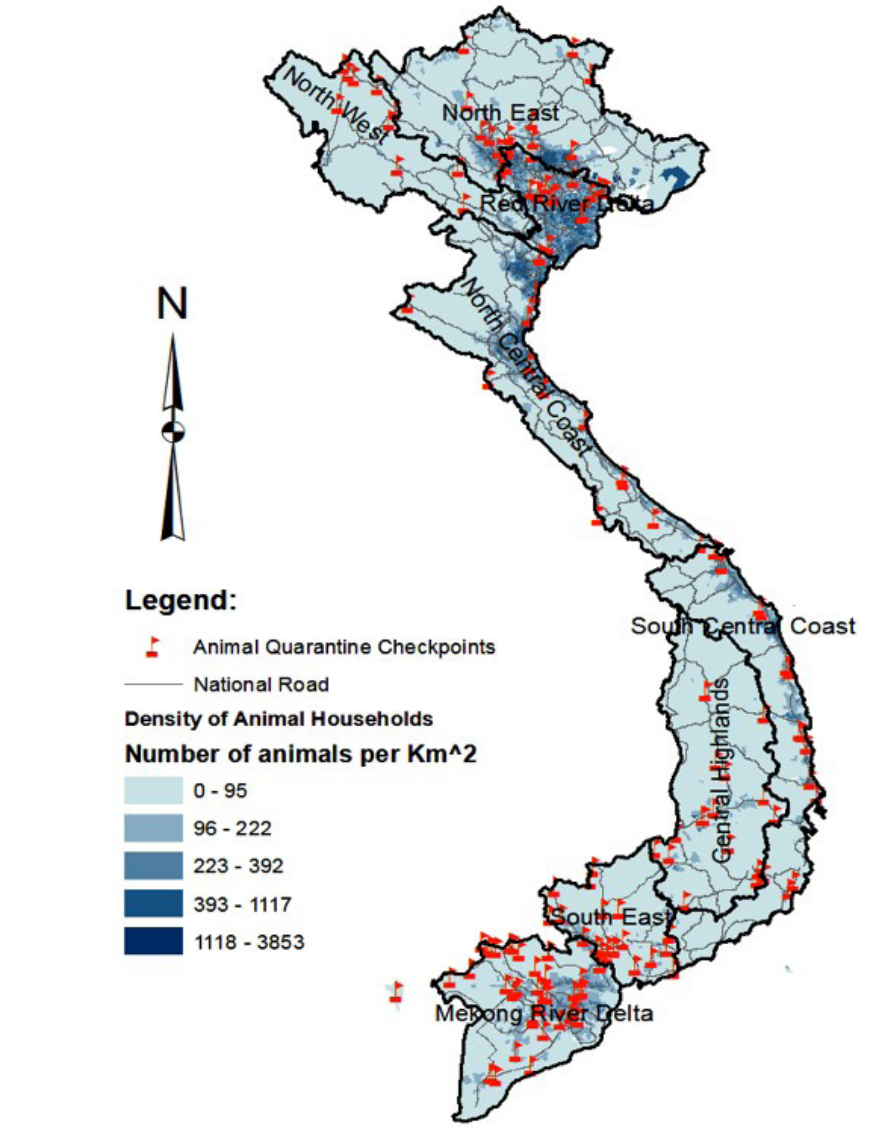

Figure 9. Quarantine of aquatic animals/products

Training programs

The government officially approved training and education plans for 630 participants per year on average at national level. At provincial level, yearly, each province organizes training for local staff and farmers on new regulations, knowledge and skills for prevention and control of aquatic animal disease.

Official training programs focused on the following topics:

- Enhancing aquatic animal disease management capacity for veterinary officials from central, regional and provincial levels. Post-graduate education at educational institutions in Viet Nam and overseas on

veterinary epidemiology (data analysis, disease warning), disease diagnosis, pathology, biosecurity, and other related topics

- Strengthening capacity of local aquatic animal health system on legislation, disease surveillance, reporting and response to disease outbreaks.

Surveillance systems (passive surveillance programs for targeted and non-targeted diseases and active surveillance programs for targeted disease)

Passive Surveillance programs

The owner of shrimp farms and aquatic animal health system implement passive surveillance following Circular number 04/2016/TT-BNNPTNT dated 10 May 2016 stipulating prevention and control of aquatic animal diseases.

Report

The owner of farming premise who observes diseased aquatic animals, aquatic animal with signs of disease, mass mortality due to disease, environment, and climate was responsible for making notification to competent authority.

Respond

Veterinary staff (field staff) in the commune, veterinary station (district level) should inform regulated level to local authority and Provincial Veterinary Office (sub-DAH), the DAH for prompt disease control.

- Outbreak investigation: inspection, take sample to test, guide farmers to treatment

- Treatment of aquatic animal during disease outbreak: Harvest, treatment (if have drugs therapies are not encourage) or disposal by chemical means and disinfection of the culture area including material, instruments, human, water and pond area.

- Declaration of epidemics, organize control of aquatic animal diseases (If needed).

- Control transport of aquatic animals in epidemic areas.

- Report

Active Surveillance programs

Base on the purpose of programs, local government or central government could implement the active surveillance program to assess prevalence in some main culture area. Active surveillance usually follows the guideline of DAH.

Additionally, if farmers/compartments want to reach certificate of disease-free, they have to follow Circular number 14/2016/TT-BNNPTNT dated 02 June 2016 stipulating animal disease-free zones and establishments. The active surveillance program will be design to meet requirement of DAH (P = 0.5 for stock company, P = 10 for shrimp production companies) or OIE's regulation (p = 0.2).

Summary and recommendation

AHPND and WSD occur year round; however, it is most frequently observed between March to September. Both the tiger shrimp and the white-leg shrimp are infected but the white-leg shrimp is more

susceptible than the other. AHPND and WSD were more severe after stocking within 15 to 60 days.

To prevent and control AHPND and WSD, Vietnam apply multi-approach including a comprehensive policy system, enhancing technique and human resources development, and improving international collaboration.

Based on the Vietnamese experiences, below are recommendations to improve aquatic animal health in Southeast Asia.

1. Provide guidance to farmers to improve their production conditions, facilities and bio-security application;

2. Improve capacity:

   - Laboratory: Improve capacity on diagnosis; organize and take part in inter-laboratory testing with OIE reference laboratory

   - Field staff: improve capacity on outbreak investigation, surveillance/study design to assess disease.

3. Build, guide and carry out early disease warning system.

4. Carry out intensive active surveillance at wide shrimp production areas for early warning.

5. Build disease-free farms especially stock farms.

6. Broodstock and post-larvae should not be accepted if positive for any OIE listed diseases.

7. Movement control to prevent spreading the disease.

References

Overview fisheries of Vietnam. http://vasep.com.vn/1192/OneContent/tong-quan-nganh.htm. Accessed June 20, 2019.

FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp, Food and Agriculture Organization of the United Nations. *Report of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304): Hanoi, Viet Nam, 25-27 June 2013.*; 2013. http://bibpurl.oclc.org/web/48266/018/i3422e/i3422e.pdf http://www.fao.org/docrep/018/i3422e/i3422e.pdf. Accessed June 20, 2019.

Tran L, Nunan L, Redman RM, *et al*. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis Aquat Org*. 2013;105(1):45-55. doi:10.3354/dao02621

N. The Hien, N. T. L. Huong, V. D. Chuong, *et al*. Status of acute hepatopancreatic necrosis disease (AHPND) and other emerging diseases of penaeid shrimps in Viet Nam. In: *Addressing Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia: Proceedings of the ASEAN 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*. Aquaculture Department, Southeast Asian Fisheries Development Center; 2016:88–95.

Boonyawiwat V, Nga NTV, Bondad MG. Risk Factors Associated with Acute Hepatopancreatic Necrosis Disease (AHPND) Outbreak in the Mekong Delta, Viet Nam. 2018:17.

OIE. *Regional Aquatic Animal Disease Yearbook - 2011*. The OIE Regional Representation for Asia and Pacific; 2012:71p., 47.

OIE. *Regional Aquatic Animal Disease Yearbook - 2012*. The OIE Regional Representation for Asia and Pacific; 2013:82p, 59-60.

Acute Hepatopancreatic Necrosis Disease (AHPND) and Hepatopancreatic Microsporidiosis (HPM): two threats to sustainable shrimp aquaculture

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Abstract

Infectious diseases caused by viruses and bacteria are a major threat to sustainable shrimp farming globally. Since early 80's viral diseases such as White Spot Disease, Taura Syndrome disease have caused enormous losses to shrimp aquaculture both in eastern and western hemisphere. As the shrimp industry tried to recover from the onslaught of these diseases, a bacterial, Acute Hepatopancreatic Necrosis Disease (AHPND), also known as Early Mortality Syndrome, and a fungal disease Hepatopancreatic Microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) are now posing new threat to shrimp aquaculture.

Acute Hepatopancreatic Necrosis Disease is caused by *Vibrio spp.* expressing plasmid-borne binary toxins, PirA and PirB that is similar to entomopathogenic bacterium, *Photorhabdus* encoded toxin. In 2009, AHPND emerged in China and since then spread to many countries in East Asia and in the Americas. Another disease that has caused alarm in recent year is Hepatopancreatic Microsporidiosis (HPM) caused by Enterocytozoon penaei (EHP), a microsporidium. While AHPND causes acute infection and large-scale mortalities, EHP causes chronic infection and results growth retardation and size variation in population reducing marketability of the infected shrimp. Both diseases affect hepatopancreas, an organ involved in metabolism and humoral immunity in shrimp. The binary toxin, PirA/ PirB are the primary virulence factor for AHPND, but specific virulence factor(s) for EHP is not known. It is, however, known that EHP does not have mitochondria and appears to transport ATP from the cytoplasm of infected cells as it contains ATP transporter genes in its genome. EHP has been shown to be a risk factor for AHPND. Due to lack of therapeutics, preventative measures remain as a corner stone for managing these diseases and efforts are underway to develop genetically improved lines of shrimp having resistance to AHPND and EHP.

Key words: AHPND, EHP, Penaeus vannamei

Introduction

The global shrimp industry has grown rapidly since it started in 1970s. Currently, shrimp aquaculture contributes to more than 50 percent of the world's production (nearly 4.5 million metric tons, MMT) (Anderson *et al*., 2018) and shrimp ranks third in the value chain of total aquaculture production after salmon and tilapia (FAO, 2020). Pacific white shrimp, *P. vannamei* and black tiger shrimp, *P. monodon* are the two most cultivated penaeid shrimp. Currently, *P. vannamei* accounts for approximately 73 % of farmed shrimp globally. The worldwide expansion of Pacific white shrimp farming has been possible due to the ease of farming this species in a wide range of salinity levels, development of captive breeding programs, and availability of genetically superior broodstock and postlarvae (PL). Unlike *P. vannamei*, captive breeding program is not well established in *P. monodon*. As a result, despite high commercial value of this species, farming of *P. monodon* remains restricted to some countries in Asia (e.g. India, Bangladesh, Thailand, Taiwan) and East Africa (e.g. Madagascar) (Thornber *et al*., 2019). As shrimp farming expanded from a backyard small farming to a major industrial operation, it faces myriads of challenge, and infectious diseases caused by viruses, bacteria and fungi remain as the primary bottleneck for further expansion of the industry. In fact, emerging diseases are now threatening the long-term sustainability of the shrimp industry worldwide.

One of the most critical issues in aquaculture is the incidence of various diseases that threaten the health of aquatic animals. Virtually, aquatic animals swim in a microbial soup that make them vulnerable to viruses, bacteria, fungi, and protozoa (Santos *et al*., 2019). In shrimp aquaculture, it is estimated that about 60 % of the losses are due to viruses and 20 % due to bacteria with the remaining losses attributed to fungi and parasites (Flegel, 2019). Viruses that have caused serious economic losses since their emergence include white-spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), shrimp hemocyte iridovirus (SHIV), yellow-head virus (YHV), and Taura syndrome virus (TSV) (Karunasagar and Ababouch, 2012; Lightner *et al*., 2012; Santos *et al*., 2019), and almost 80 % of these economic losses have occurred in Asia (Santos *et al*., 2019). Among bacterial diseases acute hepatopancreatic necrosis disease (AHPND), also known as Early Mortality Syndrome (EMS), is the most important diseases that have caused major economic losses (Tran *et al*., 2013). In fact, since its emergence in 2009 in China, AHPND has caused over USD $10 billion to shrimp aquaculture worldwide and today White spot syndrome disease AHPND are the two most economically important diseases threatening shrimp aquaculture worldwide. Among fungal disease, *Enterocytozoon hepatopenaei* (EHP), a microsporidium is the most economically important disease that has caused serious losses in recent years (Rajendran *et al*., 2016). This review will provide an overview of AHPND and EHP diagnosis and strategies to managing these diseases to prevent continued losses in shrimp aquaculture due to these diseases.

Acute Hepatopancreatic Necrosis Disease (AHPND)

Clinical signs and histopathology

The AHPND infected shrimp show pale or discoloration hepatopancreas, empty or interrupted digestive tracts. Generally, the mortality occurs within the initial 20–30 days after stocking a pond with postlarvae (PL), although there is a report that the disease can occur in late stage juveniles even at 94-day post-culture (De La Peña

et al., 2015) (**Fig. 1A**). Histopathology of AHPND susceptible shrimp primarily shows two phases of disease development defined as acute and terminal phases (Tran *et al*., 2013), and more recently, in AHPND tolerant lines a third phase, a chronic phase has been described (Aranguren *et al*., 2020c) The acute phase is characterized by the medial to distal dysfunction of HP tubule cells such as B (blister like), F (fibrillar), and R (resorptive) cells, prominent karyomegaly, and lack of mitotic activity in E cells (embryonic). In the terminal phase, the hemocytic infiltration and a secondary bacterial infection were observed in infected hepatopancreases (Lai *et al*., 2015; Lightner, 2012). In the chronic phase, melanized granuloma and hemocytic nodules in the HP that resembles septic hepatopancreatic necrosis (SHPN) are observed (Aranguren *et al*., 2020c; Mai *et al*., 2021) (**Figure1B**).

Geographical distribution

Acute Hepatopancreatic Necrosis Disease emerged in China in 2009 (Lightner and Flegel, 2012). Since then, AHPND was reported in several shrimp producing countries from Asia to Latin America such as Viet Nam (Tran *et al*., 2013), Malaysia in 2011 (Lightner and Flegel, 2012), Thailand in 2012 (Joshi *et al*., 2014), Mexico in 2013 (Nunan *et al*., 2014), the Philippines in 2014 (De La Peña *et al*., 2015), and more recently in Bangladesh (Eshik *et al*., 2017), Myanmar (Lai *et al*., 2015), USA (in Texas, 2017)(Dhar *et al*., 2019), South Korea in 2019 (Han *et al*., 2020) and Japan in 2020 (OIE, 2021)(**Figure 2**).

Etiology

The etiologic agent of AHPND was originally shown to be a specific strain of *Vibrio parahaemolyticus* called the AHPND-causing *V. parahaemolyticus* (VP_{AHPND}) (Han *et al*., 2017; Lee *et al*., 2015). *Vibrio parahaemolyticus*, is a Gram-negative, halophilic, rod-shaped bacterium which is widely present in marine environments. *Vibrio parahaemolyticus* becomes virulent after acquiring a plasmid (pVA1) that expresses a deadly binary toxin Pir^{vp} (Dong *et al*., 2017). The toxin consists of two subunits, $PirA^{vp}$ and $PirB^{vp}$, and is homologous to the Pir (*Photorhabdus* insect-related) binary toxin (Dong *et al*., 2017). The plasmid pVA1 that carries the toxin genes is 69-73 kb in size and was found to contain a cluster of genes related to conjugative transfer indicating that the

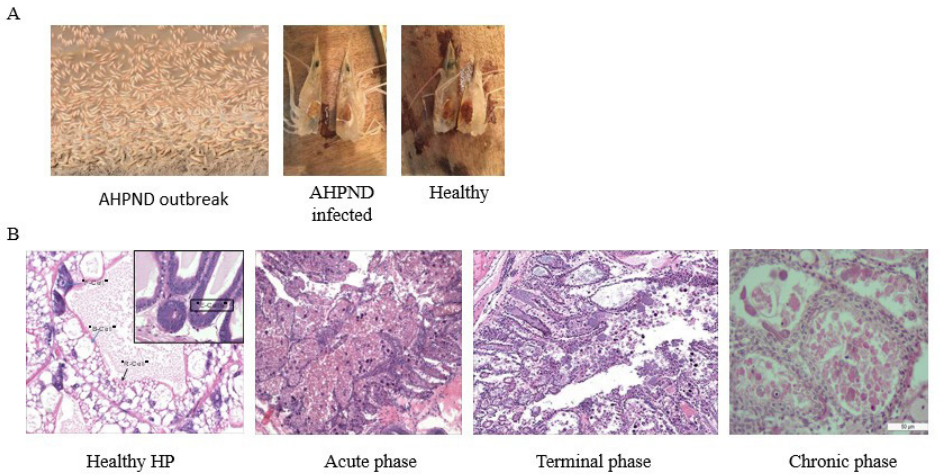

Figure 1. Clinical signs and histopathology of AHPND. (A) Clinical signs of AHPND and (B) Histopathology of AHPND (Mai *et al*., 2021)

Figure 2. Graphical distribution of AHPND. Arrows and colors indicate the countries (Tang *et al.*, 2020)

plasmid may potentially be able to transfer not only among *V. parahaemolyticus* strains, but also to different bacterial species (Lee *et al*., 2015). In fact, since the isolation of AHPND-causing *V. parahaemolyticus*, number of other *Vibrio* species causing AHPND were isolated including *V. harveyi*, *V. campbellii*, *V. owensii*, and *V. punensis* (Dong *et al*., 2017; Han *et al*., 2017; Kondo *et al*., 2015; Restrepo *et al*., 2018). The tertiary structure of $PirA^{vp}$ and $PirB^{vp}$ toxins have similarity to Cry insecticidal toxin-like protein that has a pore-forming activity leading to cell deaths in insects (Prachumwat *et al*., 2019). It is believed that AHPND-causing *Vibrio parahaemolyticus* colonizes shrimp stomach and releases binary toxin which enters hepatopancreas via the gastric sieve. The molecular mechanisms by which toxins induce cell necrosis and severe sloughing in hepatopancreas tissue in shrimp are yet to be determined.

Recently, Aranguren and colleagues identified two novel isolates of *V. parahaemolyticus* from Latin America while screening these isolates to detect binary toxin genes, *pirAB(+)* by PCR (Aranguren *et al*., (2020b). These authors reported a unique stain of *V. parahaemolyticus* (i.e., R13) that carries only *pirA* and not *pirB* while there was a second strain (i.e., R14) that carries both *pirAB* (+) genes. Interestingly, neither strain caused clinical signs of AHPND in experimental bioassay including the strain R14 that contains both binary toxin genes (Aranguren *et al*., (2020b). Genomic analysis revealed a complete deletion of *pirA* gene in R13 resulting in loss of virulence. It is possible that the deletion of *pirA* is mediated by transposase elements (**Figure 3**) which may play a role in transferring toxin genes between cells through conjugation or plasmid uptake (Lee *et al*., 2015; Tang *et al*., 2020). In the R14 strain, there is an insertion of transposase element upstream of the promoter region of *pirA* and *pirB* genes. Although insertion of transposase element did not disrupt transcription of these genes, translation of $PirA^{vp}$ and $PirB^{vp}$ toxins was inhibited as determined by western blot analysis using polyclonal antibodies against $PirA^{vp}$ and $PirB^{vp}$ toxins (Aranguren *et al*., 2020b) (**Figure 3**). This finding has a major implication in PCR-based screening of *Vibrio* isolates. Because R14 is tested positive for pirA and pirB genes by duplex PCR following OIE-recommended protocol for screening AHPND-causing *Vibrio spp.* The finding suggests DNA based detection such as PCR is not enough to conclude the pathogenicity of AHPND-causing *Vibrio* spp., and experimental bioassay needs to be conducted to delineate the pathogenic potential of the bacterial isolates(s).

In addition, genomic comparison reveals that the genome of AHPND and non-AHPND causing *V. parahaemolyticus* strains were 50-100 % identical to clinical strain of *V. parahaemolyticus* (**Figure 4**). It is worth noting that like other Gram negative bacteria, *V. parahaemolyticus* also deploys two conserved secretory systems (T3SS1 and T3SS2) to deliver virulence factors into the cytoplasm of the infected host cells (Galán *et al*., 2014). The function of T3SS1 and T3SS2 as a secretary system and its role in pathogenicity was demonstrated by Park and colleagues using a series of deletion mutants *V. parahaemolyticus* (Park *et al*., 2004). The pVA1 plasmid contains two type-II secretion systems (T2SSs), two type-III secretion systems (T3SS1 and T3SS2), two type-IV secretion systems (T6SS1, T6SS2), and two T2/4SSs (Gomez-Gil *et al*., 2014; Henke and Bassler, 2004). Interestingly, the genes responsible for T3SS1 in A3 strain (AHPND causing strain) are different from the genes in T3SS1 in non-AHPND causing strains. It is widely

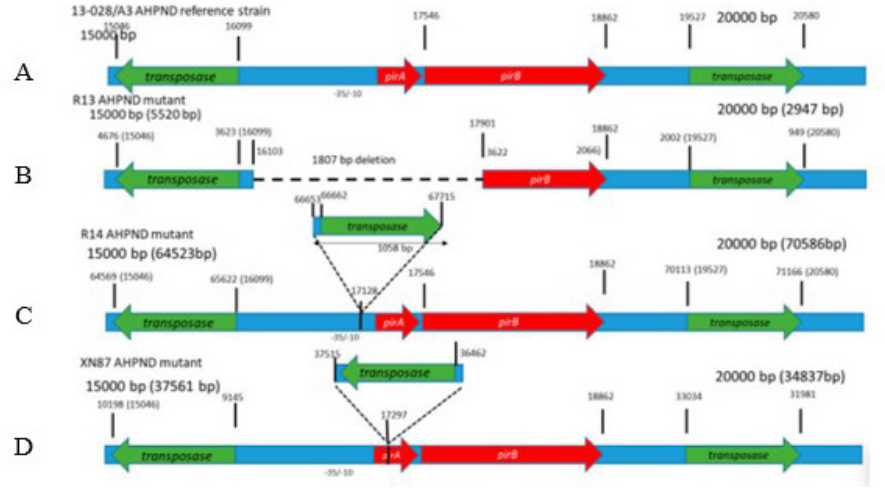

Figure 3. Comparison of the normal pVA3 plasmid (strain A3) and their mutated forms in isolate R13 and R14. (A) Scaled diagram of the pirA and pirB toxin gene regions in the normal pVA3 plasmid. (B) Mutant strain R13. Dash lines denote the absence of pirA. (C) Mutant strain R14. Notice insertion upstream of pirA. (D) XN87 AHPND mutant. The numberx over the gene organization schematics indicate the nucleotide position (Aranguren et al., 2020)

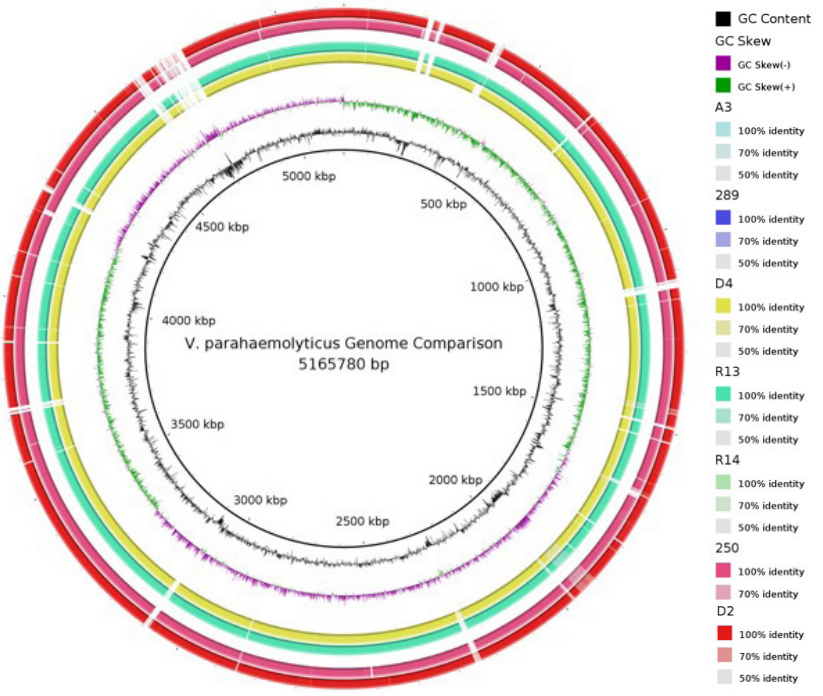

Figure 4. Genomic comparison between AHPND and non-AHPND *V. parahaemolyticus* and *V. parahaemolyticus* clinical strain RMID2210663. The graph was generated by Blast Ring Image Generator (BRID)

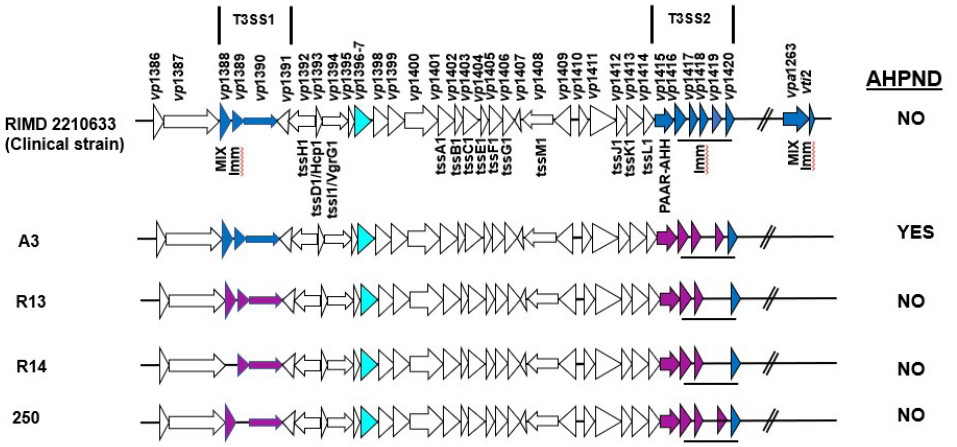

Figure 5. Comparison of genomic architecture of pathogenicity island between *V. parahaemolyticus* clinical strains versus non-AHPND and AHPND causing *V. parahaemolyticus*. Same genes were indicated by same colors.

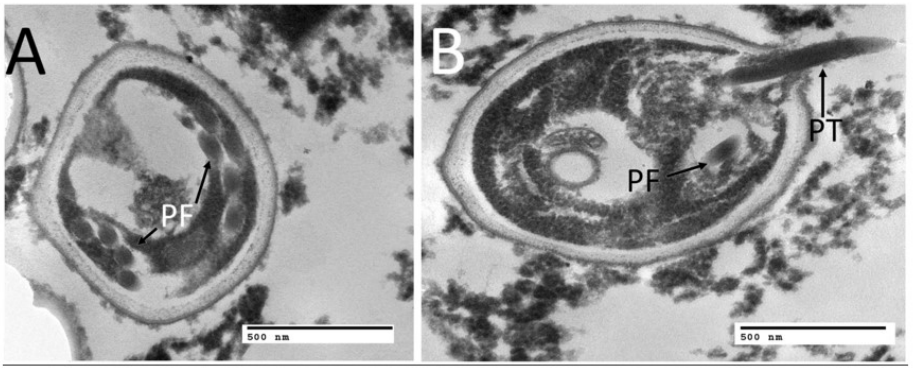

Figure 6. Electron microscopy of *Enterocytozoon hepatopenaei* from the pelleted biopsy samples. (A) Mature spore showing a section of the coiled part of the polar filament (PF), five coils are observed on one side and four coils are observed on the other side. (B) Cross section of a mature spore were a coiled portion of the PF and the polar tube (PT) are observed. Magnification of both images is 43,000× (Cruz-flores et al., 2019)

accepted that the binary toxin PirAB*vp* is a secreted protein, thus, the secretion systems, especially T3SS, found in pVA1 may contribute to the virulence of AHPND causing strains.

Diagnosis

AHPND is a threat to sustainability of global shrimp aquaculture. Therefore, early detection via screening of broodstock and post larvae, shrimp feed and feed ingredients are necessary to prevent further spread and disease outbreaks. Since 2013, several methods have been developed to detect AHPND. Initially, AHPND has been detected by histopathology (Tran *et al*., 2013) but histopathology alone is not enough to unequivocally confirm AHPND. Later several molecular methods were developed to detect AHPND. Detection of

AHPND using molecular method initially targeted pVA1 plasmid as a marker (Lo and Flegel, 2014). However, when pirA and pirB genes encoding binary toxin, PirAB were identified as virulent factors in *V. parahaemolyticus*, efforts were made to develop a PCR-based assay to detect the toxin genes causing AHPND (Han *et al*., 2015b). Since then, pirA and pirB genes have become molecular hallmark of AHPND detection. Both conventional PCR (Dangtip *et al*., 2015; Han *et al*., 2015b; Tinwongger *et al*., 2014) and real-time PCR using *Taq*Man chemistry (Cruz-Flores *et al*., 2019a; Han *et al*., 2015a) and SYBR green dye (Cruz-Flores *et al*., 2019a; Han *et al*., 2015a) were developed for the detection of AHPND. More recently, point-of-care diagnostic methods including a loop-mediated isothermal amplification (LAMP) and a recombinase polymerase amplification (RPA)-based detection methods have been reported for AHPND detection at a pond-site (Koiwai *et al*., 2016; Mai *et al*., 2021). Apart from DNA based detection, polyclonal and monoclonal antibodies against PirA and PirB toxins have been generated to detect AHPND via ELISA and western-blot assays (Mai *et al*., 2020b; Wangman *et al*., 2017).

Hepatopancreatic Microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei*.

Hepatopancreatic microsporidiosis

Unlike many diseases causing large-scale mortalities, retarded growth syndrome receives less attention until the economic impact of this syndrome is reported. For example, in 2002 in Thailand the losses caused by retarded growth syndrome was estimated as much as $3M (Chayaburakul *et al*., 2004). Although there are several microbial agents reported to be associated with retarded growth

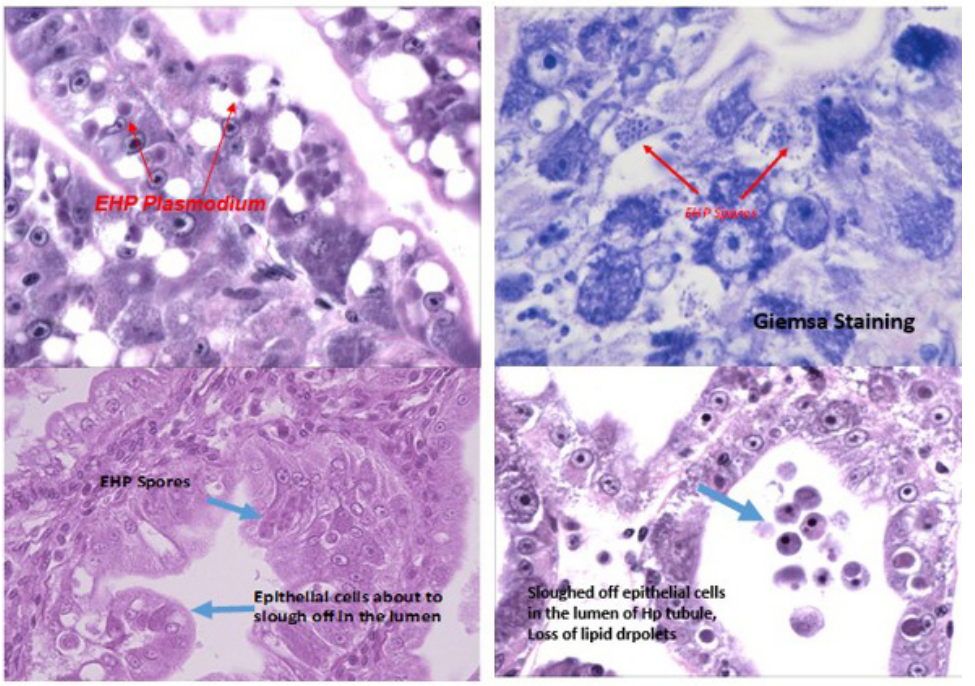

Figure 7. Histopathology of EHP infected Hepatopancrease

syndrome, a microsporidian species called *Enterocytozoon hepatopenaei* (EHP) was found to be involved with retarded growth syndrome in *P. vannamei* shrimp (Chayaburakul *et al*., 2004; Tourtip *et al*., 2009). Microsporidia were first identified in silkworm as a causal agent of Pebrine disease in domesticated silkworm 150 years ago (Han and Weiss, 2017). Microsporidia are intracellular parasite and has now been reported from an wide array of invertebrate and vertebrate species including *Nosema apis* and *Nosema caeranae* in honeybee, *Loma salmonae* in Salmon *Oncorhynchus kisutch* and EHP in shrimp (Fries, 1993; Kent *et al*., 1989; Tourtip *et al*., 2009). Although EHP has been identified since 2009, the potential threat of the parasite was largely ignored due to the impact of AHPND. However, EHP soon emerged as a threat for its negative impact on shrimp production.

Biology and pathogenicity of EHP

EHP was first reported from Thailand in 2009 and found to be associated with retarded growth syndrome *P. vannamei* grow-out ponds. Since then, EHP has been detected in most of retarded growth syndrome pond from other shrimp producing countries including Vietnam, India, Indonesia, Malaysia, China in Asia, and Venezuela in the western hemisphere. EHP is known to infect several shrimp species including *P. monodon*, *P. vannamei*, *P. stylirostris* (Tang *et al*., 2015). EHP was also detected in artemia (Tang *et al*., 2015) although pathogenicity of EHP to artemia has not been demonstrated. It is and presumed to serve as a carrier of the parasite in shrimp hatchery. EHP has been detected in *P. vannamei* cultured in high and low salinities although the severity of infection is higher in high salinity (30 ppt) than low salinity (2 and 15 ppt) (Aranguren *et al*., 2021). Since EHP was detected in a wide range of salinities, it is speculated that there are probably other host species of EHP apart from marine penaeid shrimp species. In laboratory challenge assays, EHP was successfully transmitted through oral administration, reverse gavage and via direct injection of the inoculum into hepatopancreas (Mai *et al*., 2020a). Moreover, EHP has been reported to be associated with *P. vannamei* grow-out ponds displaying white feces syndrome (WFS) (**Figure 8A**). The prevalence of EHP was found to be higher in WFS ponds compared to ponds displaying no WFS (**Figure 8B**) (Aranguren *et al*., 2020a). In addition, using a case-control study, it has been reported that EHP is a risk factor for AHPND since shrimp pre-infected with EHP exhibited higher mortality compared to uninfected shrimp (Aranguren *et al*., 2017).

In the natural environment, EHP exists as mature spores. The life cycle of EHP is not known completely. However, based on information in the published literatures and analogy to the life cycle of other microsporidia where different stages are well known, a tentative life cycle of EHP can be drawn. Most of microsporidian species start infecting host cells by using the extruded polar tube puncturing the plasma membrane of host cells and transfer the content of the spore, the sporoplasm into host cell cytoplasm. Then, the sporoplasm develops into a plasmodium. Once the extrusion precursors (i.e., polar filament and anchoring dish) are generated, the cytoplasm of plasmodium is cleaved to sporoblast. Finally, the sporoblast develops into mature spores which are released and infect other cells (Chaijarasphong *et al*., 2020). EHP spores are unicellular containing coiled polar filaments with the size of 1.1 ± 0.2 µm × 0.6 ± 0.2 µm(Cruz-Flores *et al*., 2019b; Tourtip *et al*., 2009). The spores have shared a common ultrastructure with other microsporidian

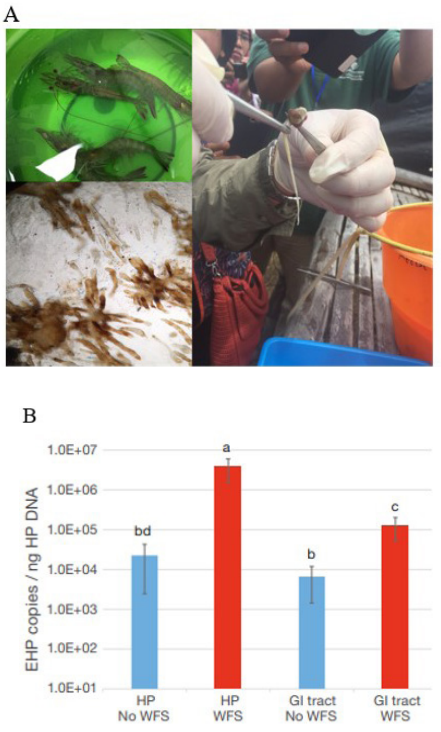

Figure 8. The association of EHP and White feces syndrome (WFS). (A) Clinical sign of WFS. (B) EHP quantification in Hepatopancreases (HP) and GI tracts from different types of samples

spores in containing a plasma membrane covered by two electron-dense layers, and a nucleus surrounded by polar tube coils (**Figure 6**). Histopathology of EHP-infected hepatopancreas reveals epithelial cell sloughing into lumen of hepatopancreatic tubules, and spores and plasmodium could be seen in the detached cells in the lumen (**Figure 7**). A draft genome sequence of EHP has been published and a putative spore wall protein (SPW) gene has been identified although its function remains unknow. It is presumed that, like other microsporidia, SWP of EHP enables spore to maintain its shape and withstand environmental stress. Since SWP represents the outer layer of spore, it may be involved in some critical steps in EHP pathogenesis including adherence to host cells and spore extrusion.

Diagnosis

The current standard for detection of EHP include histopathology, *in situ* hybridization (ISH), and PCR. The use of gross clinical signs is not typically very reliable due to the EHP infection requiring around 30 to 45 days to cause slow growth syndrome. Initially, H&E histology was developed to detect EHP. The H&E of EHP infected HP is characterized as the presence of EHP life stages and mature spores in the tubule epithelial cells and the sloughing off of epithelial cells (Aranguren *et al*., 2017). However, the sensitivity of H&E method does not meet the requirement in EHP prevention strategy. Moreover, the result of histopathology needs to be confirmed by *in situ* hybridization (ISH). The DIG-labelled 18SrRNA gene probe is used in *in situ* hybridization assay to detect EHP. *In situ* hybridization enables to determine the severity of EHP infection because all life stages of EHP from meront to mature spore can be detected even with a low magnification (Tang *et al*., 2015). However, ISH is a technically challenge method, takes long time and is not feasible to use as a routine method to detect EHP in laboratory where resources are limited.

Molecular detection methods were developed based on EHP 18S rRNA or SSU rRNA gene sequence (Tang *et al*., 2015) (**Figure 7** and **Figure 9**). Using PCR EHP can be successfully detected in different samples including *P. vannamei* tissue, feces, artemia and contaminated water (**Figure 9A**). However, PCR assay for EHP detection has been problematic as it gives false positive results with the samples collected from environment. It was shown that the annealing site of primer developed by Tangprasittipap and colleagues (Tangprasittipap *et al*., 2013) has 66.7 %–90 % identity to that of other microsporidia resulting in false positive result. Subsequently, Jaroenlak *et al*.,

(2016) and Han *et al*., (2018) developed nested PCR based on the sequence of spore wall protein and □-tubulin genes. The specificities of the later methods are higher than method where primers are designed based on SSU rRNA sequence.

Apart from conventional PCR, *Taq*Man-probe based qPCR for EHP quantification also has been developed (Liu *et al*., 2018). The primers and probes used in real-time PCR assay are designed based on SSU rRNA gene sequence. There is a need to further improve the specificity and sensitivity of the currently available real-time PCR for EHP quantification considering occasional spurious results are obtained while using feed and feed ingredients for EHP screening (Dhar *et al*., unpublished).

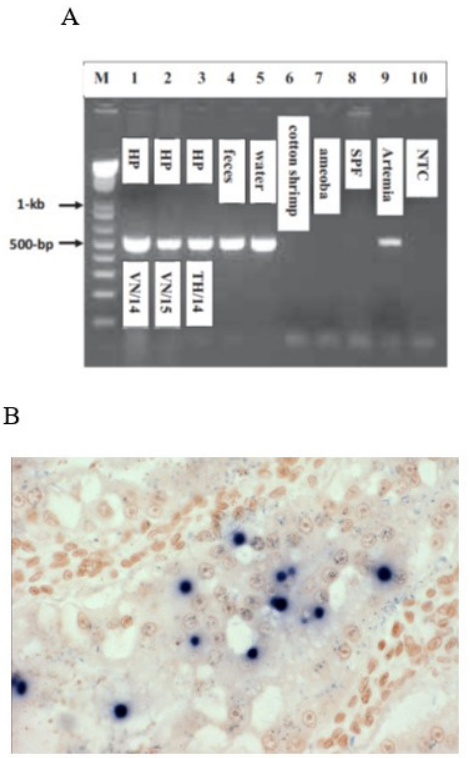

Figure 9. EHP detection by PCR based on 18S rRNA gene. (A) EHP detection in several samples by PCR. (B) EHP detection by in situ hybridization method. Dark blue spots indicated positive reactions

EHP can be transmitted via contaminated water, and it is presumed that EHP can also be transmitted from broodstock to offspring. Therefore, broodstock screening is recommended to prevent EHP spread in the hatchery. Sacrificing broodstock is a very expensive proposition. Recently, Cruz-Flores *et al*. (2019b) developed an invasive but non-lethal method for sampling hepatopancreas for EHP detection (**Figure 10**). Using hepatopancreas biopsy tissue, EHP was detected by real-time PCR and transmission electron microscopy. Since often EHP is not uniformly present throughout the hepatopancreas tissue, it remains to be determined if EHP detected by conventional PCR detection method using an aliquot of entire hepatopancreas homogenate is comparable to tissue biopsy to determine the feasibility of hepatopancreas biopsy as a means of EHP screening of EHP.

Management of AHPND and HPM

It is well known that shrimp diseases like AHPND and HPM need to be controlled in hatchery and grow-out pond levels. Considering the lack of a therapeutic, bio-security remains as a most efficient approach to control AHPND causing *Vibrio* sp. and EHP causing. At a hatchery level, broodstock should be routinely screened for *Vibrio spp*. containing *pir*A/*pir*B genes and EHP. Similarly, before introducing new stock to enhance genetic diversity in a captive breeding program, the new stock should be screened while in a quarantine facility prior. In addition, EHP can be introduced in a hatchery via contaminated feed such as live polychaetes. Thus, live polychaetes and potentially other live feed should be screened for EHP and AHPND prior to feeding broodstock. Recently, Mai *et al*., (2020a) and Munkongwongsiri *et al*., (2021) showed EHP can be inactivated by either freezing at -20 ^{0}C or boiling at

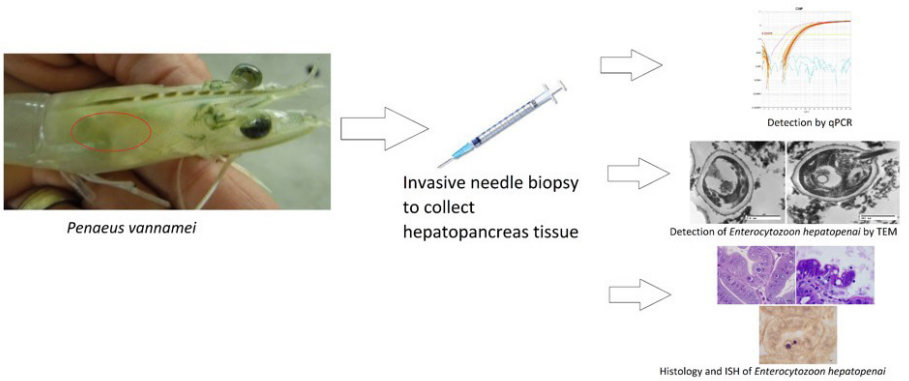

Figure 10. A schematic overview of EHP detection using invasive non-lethal sampling method. (Cruz-Flores et al., 2019)

75 ^{0}C for 1 minute. Therefore, due to lack of formulated feed, feeding live polychaetes to broodstock should be avoided and instead freezing or boiling polychaetes is recommended. In grow-out ponds, the post-larvae (PL) should be screened for the presence of *pir*A/*pir*B toxin genes and EHP prior to stocking. In addition, *pir*A/*pir*B and EHP can be detected in water and sediment thus reducing the buildup of organic waste by water exchange/ treatment is recommended to reduce the risk of AHPND and HPM. There is a need to develop AHPND and HPM resistant/ tolerant shrimp to prevent the further spread of these diseases. In endemic area, to minimize the losses due to these diseases stocking of ponds with tolerant/ resistant lines are needed. In nature, host–pathogen interaction is considered as a highly dynamic process, and when the interaction is tipped, disease outbreak occurs (**Figure 11**). Understanding the biological and environmental factors that modulate this balance is critical to avoid disease outbreaks.

Future direction in AHPND and HPM research

It has now over a decade that shrimp aquaculture has been affected by AHPND and HPM. For the past decade, considerable efforts have been made to understanding the biology of these diseases and developing tools to diagnose these diseases and preventing their global spread. While these progresses have been instrumental in thwarting the negative impact of these diseases, there are still major gaps in understanding the pathogenesis of these diseases. For example, the receptors of AHPND toxin have not been fully elucidated. Identifying these receptors will contribute to our understanding of disease development at a molecular and cellular levels. This may also aid in developing therapeutics to control AHPND since use of antibiotics to control AHPND is not a feasible option due to the development of antimicrobial resistance. methods to the end of AHPND outbreaks through AHPND toxin receptors blocking. However, controlling AHPND by utilizing AHPND receptors might be challenging because AHPND toxin may utilize more than receptor, and those receptors may play important roles in basic cellular processes. Understanding the molecular and cellular basis of AHPND is certainly a fertile area of future research. Recently, we have identified a genetic line of *P. vannamei* shrimp that shows resistance to AHPND (Aranguren *et al*., 2020c). Such a line could be a valuable genetic resource to identify marker(s) for AHPND resistance/

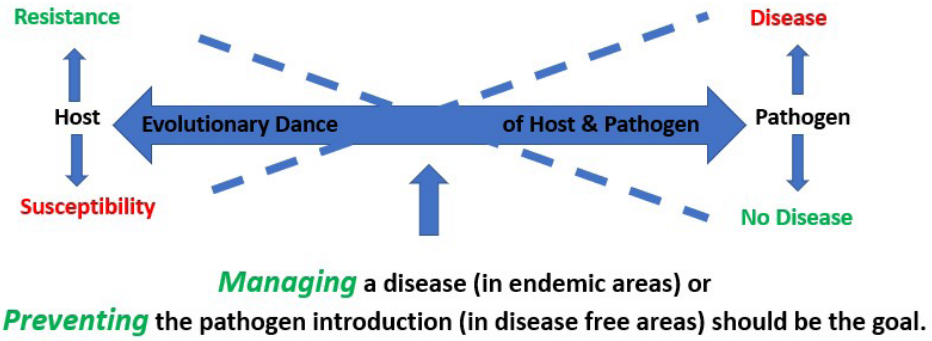

Figure 11. A schematic overview of host-pathogen evolution in shrimp diseases

susceptibility. Similarly, the role of EHP in white feces syndrome has not been demonstrated unequivocally. Therefore, identifying microbiological factors such as involvement of bacteria or environmental factors needs to be determined. Availability of an experimental model to reproduce white feces syndrome at a laboratory level is urgently needed to screen genetic lines that are resistant to HPM and whit feces syndrome.

Acknowledgement

The authors would like to acknowledge Dr. L. Fernando Aranguren Caro, Ms. Jasmine Millabas, Brenda Noble, Paul Schofield and Tanner Padilla for their help in performing many AHPND and HPM studies. This work was supported by The University of Arizona-Aquaculture Pathology Laboratory. This work is/was supported by the USDA National Institute of Food and Agriculture, Animal Health project 1024420 to AKD.

References

Anderson, B.J.L., Valderrama, D., Jory, D.E. 2018. Global shrimp production review and forecast : Steady growth ahead. Glob. Aquac. Alliance 5–10.

Aranguren, Alghamdi, F., De Belder, K., Lin, J., Mai, H.N., Millabas, J., Alrehaili, Y., Alazwari, A., Algetham, S., Dhar, A.K. 2021. The effect of salinity on *enterocytozoon hepatopenaei* infection in Penaeus vannamei under experimental conditions. BMC Vet. Res. 17. https://doi.org/10.1186/s12917-021-02778-0

Aranguren, Han, J.E., Tang, K.F.J. 2017. *Enterocytozoon hepatopenaei* (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN) in the Pacific white shrimp Penaeus vannamei. Aquaculture 471, 37–42. https://doi.org/10.1016/j.aquaculture.2016.12.038

Aranguren, Mai, H., Pichardo, O., Cruz-Flores, R., Hanggono, B., Dhar, A. 2020a. Evidences supporting *Enterocytozoon hepatopenaei* association with white feces syndrome in farmed *Penaeus vannamei* in Venezuela and Indonesia. Dis. Aquat. Org. 141, 71–78. https://doi.org/10.3354/dao03522

Aranguren, Mai, H.N., Kanrar, S., Cruz-Flores, R., Dhar, A.K. 2020b. A mutant of *Vibrio parahaemolyticus pir*AB*vp* (+) that carries binary toxin genes but does not cause acute hepatopancreatic necrosis disease. Microorganisms 8, 1–13. https://doi.org/10.3390/microorganisms8101549

Aranguren, Mai, H.N., Noble, B., Dhar, A.K. 2020c. Acute hepatopancreatic necrosis disease (VP_{AHPND}), a chronic disease in shrimp *Penaeus vannamei* population raised in latin America. J. Invertebr. Pathol. 174, 107424. https://doi.org/10.1016/j.jip.2020.107424

Chaijarasphong, T., Munkongwongsiri, N., Stentiford, G.D., Aldama-Cano, D.J., Thansa, K., Flegel, T.W., Sritunyalucksana, K., Itsathitphaisarn, O. 2020. The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): Biology, pathology, diagnostics and control. J. Invertebr. Pathol. 107458. https://doi.org/10.1016/j.jip.2020.107458

Chayaburakul, K., Nash, G., Pratanpipat, P., Sriurairatana, S., Withyachumnarnkul, B. 2004. Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand. Dis. Aquat. Organ. 60, 89–96. https://doi.org/10.3354/dao060089

Cruz-Flores, R., Mai, H.N., Dhar, A.K. 2019a. Multiplex SYBR Green and duplex *Taq*Man real-time PCR assays for the detection of *Photorhabdus* Insect-Related (*Pir*) toxin genes *pirA* and *pir*B. Mol. Cell. Probes 43, 20–28. https://doi.org/10.1016/j.mcp.2018.12.004

Cruz-Flores, R., Mai, H.N., Noble, B.L., Schofield, P.J., Dhar, A.K. 2019b. Detection of *Enterocytozoon hepatopenaei* using an invasive but non-lethal sampling method in shrimp (Penaeus vannamei). J. Microbiol. Methods 162, 38–41. https://doi.org/10.1016/j.mimet.2019.05.008

Dangtip, S., Sirikharin, R., Sanguanrut, P., Thitamadee, S., Sritunyalucksana, K., Taengchaiyaphum, S., Mavichak, R., Proespraiwong, P., Flegel, T.W. 2015. AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio* parahaemolyticus. Aquac. Reports 2, 158–162. https://doi.org/10.1016/j.aqrep.2015.10.002

De La Peña, L.L.D., Cabillon, N.A.R.N., Catedral, D.D.D., Amar, E.E.C., Usero, R.R.C., Monotilla, W.W.D., Calpe, A.A.T., Fernandez, D.D.D.G., Saloma, C.P.C. 2015. Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. Dis. Aquat. Organ. 116, 251–254. https://doi.org/10.3354/dao02919

Dhar, A.K.A., Piamsomboon, P., Aranguren Caro, L.F.L., Kanrar, S., Adami, R., Juan, Y.-S.Y. 2019. First report of acute hepatopancreatic necrosis disease (AHPND) occurring in the USA. Dis. Aquat. Organ. 132, 241–247. https://doi.org/10.3354/dao03330

Dong, X., Wang, H., Xie, G., Zou, P., Guo, C., Liang, Y., Huang, J. 2017. An isolate of *Vibrio* campbellii carrying the pir*VP* gene causes acute hepatopancreatic necrosis disease. Emerg. Microbes Infect. https://doi.org/10.1038/emi.2016.131

Eshik, M., Abedin, M., Punom, N., Begum, M., Rahman, M. 2017. Molecular Identification of AHPND Positive *Vibrio Parahaemolyticus* causing an outbreak in South-West Shrimp Farming Regions of Bangladesh. J Bangladesh Acad Sci 41, 127–135. https://doi.org/https://doi.org/10.3329/jbas.v41i2.35492

FAO. 2020. FAO Fisheries and Aquaculture Circular FIAA/C1187 (En) shrimp infectious myonecrosis strategy manual. Food Agric. Organ. United Nations.

Flegel, T.W. 2019. A future vision for disease control in shrimp aquaculture. J. World Aquac. Soc. 50, 249–266. https://doi.org/10.1111/jwas.12589

Fries, I. 1993. Bee World NOSEMA APIS-A PARASITE IN THE HONEY BEE COLONY. Bee World 74, 5–19. https://doi.org/10.1080/0005772X.1993.11099149

Galán, J.E., Lara-Tejero, M., Marlovits, T.C., Wagner, S. 2014. Bacterial type III secretion systems: Specialized nanomachines for protein delivery into target cells. Annu. Rev. Microbiol. https://doi.org/10.1146/annurev-micro-092412-155725

Gomez-Gil, B., Soto-Rodríguez, S., Lozano, R., Betancourt-Lozano, M., 2014. Draft genome sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of shrimps in Mexico. Genome Announc. 2, 55–69. https://doi.org/10.1128/genomeA.00055-14

Han, B., Weiss, L. 2017. Microsporidia: Obligate Intracellular Pathogens Within the Fungal Kingdom, in: The Fungal Kingdom. American Society of Microbiology, pp. 97–113. https://doi.org/10.1128/microbiolspec.funk-0018-2016

Han, J., Choi, S., Han, S., Chan, Seung, Jin, H., Lee, C., Yeon, K., Seo, Y., Chan, Seul, Rhee, G., Young, S., Kim, J., Park, S., Hyung, J., Lee, K. 2020. Genomic and histopathological characteristics of *Vibrio parahaemolyticus* isolated from an acute hepatopancreatic necrosis disease outbreak in Pacific white shrimp (*Penaeus vannamei*) cultured in Korea. Aquaculture 524, 735284. https://doi.org/10.1016/j.aquaculture.2020.735284

Han, J.E., Tang, K.F.J., Kim, J.H. 2018. The use of beta-tubulin gene for phylogenetic analysis of the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) and in the development of a nested PCR as its diagnostic tool. Aquaculture 495, 899–902. https://doi.org/10.1016/j.aquaculture.2018.06.059

Han, Tang, K.F.J., Aranguren, L.F., Piamsomboon, P. 2017. Characterization and pathogenicity of acute hepatopancreatic necrosis disease natural mutants, pirAB*vp*(+) *V. parahaemolyticus*, and pirAB*vp*(+) *V. campbellii* strains. Aquaculture 470, 84–90. https://doi.org/10.1016/j.aquaculture.2016.12.022

Han, Tang, K.F.J., Pantoja, C.R., White, B.L., Lightner, D. V. 2015a. QPCR assay for detecting and quantifying a virulence plasmid in acute hepatopancreatic necrosis disease (AHPND) due to pathogenic *Vibrio parahaemolyticus*. Aquaculture 442, 12–15. https://doi.org/10.1016/j.aquaculture.2015.02.024

Han, Tang, K.F.J., Tran, L.H., Lightner, D.V. 2015b. *Photorhabdus* Insect-Related (Pir) Toxin-Like Genes in a Plasmid of *Vibrio parahaemolyticus*, the Causative Agent of Acute Hepatopancreatic Necrosis Disease (AHPND) of Shrimp. Dis. Aquat. Organ. 113, 33–40. https://doi.org/10.3354/dao02830

Henke, J.M., Bassler, B.L. 2004. Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. J. Bacteriol. 186, 3794–3805. https://doi.org/10.1128/JB.186.12.3794-3805.2004

Jaroenlak, P., Sanguanrut, P., Williams, B.A.P., Stentiford, G.D., Flegel, T.W., Sritunyalucksana, K., Itsathitphaisarn, O. 2016. A Nested PCR Assay to Avoid False Positive Detection of the Microsporidian *Enterocytozoon hepatopenaei* (EHP) in Environmental Samples in Shrimp Farms. PLoS One 11, e0166320. https://doi.org/10.1371/journal.pone.0166320

Joshi, J., Srisala, J., Truong, V.H., Chen, I.T., Nuangsaeng, B., Suthienkul, O., Lo, C.F., Flegel, T.W., Sritunyalucksana, K., Thitamadee, S. 2014. Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). Aquaculture 428–429, 297–302. https://doi.org/10.1016/j.aquaculture.2014.03.030

Karunasagar, I., Ababouch, L. 2012. Shrimp viral diseases, import risk assessment and international trade. Indian J. Virol. 23, 141–148. https://doi.org/10.1007/s13337-012-0081-4

Kent, M.L., Elliott, D.G., Groff, J.M., Hedrick, R.P. 1989. Loma salmonae (Protozoa: Microspora) Infections in Seawater Reared Coho Salmon *Oncorhynchus kisutch*, Aquaculture. Elsevier Science Publishers B.V.

Koiwai, K., Tinwongger, S., Nozaki, R., Kondo, H., Hirono, I. 2016. Detection of acute hepatopancreatic necrosis disease strain of *Vibrio parahaemolyticus* using loop-mediated isothermal amplification. J. Fish Dis. 39, 603–6. https://doi.org/10.1111/jfd.12387

Kondo, H., Van, P.T., Dang, L.T., Hirono, I. 2015. Draft Genome Sequence of Non-*Vibrio parahaemolyticus* Acute Hepatopancreatic Necrosis Disease Strain KC13.17.5, Isolated from Diseased Shrimp in Vietnam. Genome Announc. 3. https://doi.org/10.1128/genomeA.00978-15

Lai, H.C., Ng, T.H., Ando, M., Lee, C. Te, Chen, I.T., Chuang, J.C., Mavichak, R., Chang, S.H., Yeh, M. De, Chiang, Y.A., Takeyama, H., Hamaguchi, H. o., Lo, C.F., Aoki, T., Wang, H.C. 2015. Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. Fish Shellfish Immunol. 47, 1006–1014. https://doi.org/10.1016/j.fsi.2015.11.008

Lee, C. Te, Chen, I.T., Yang, Y.T., Ko, T.P., Huang, Y.T., Huang, J.Y., Huang, M.F., Lin, S.J., Chen, C.Y., Lin, S.S., Lightner, D. V., Wang, Han Ching, Wang, A.H.J., Wang, Hao Ching, Hor, L.I., Lo, C.F. 2015. The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin. Proc. Natl. Acad. Sci. U. S. A. 112, 10798–10803. https://doi.org/10.1073/pnas.1503129112

Lightner, D., Flegel, T. 2012. Diseases of Crustaceans–Acute hepatopancreatic necrosis syndrome (AHPNS)(disease card). asia pacific emergency regional consultation on early mortality syndrome (EMS)/Acute hepatopancreatic necrosis syndrome (AHPNS).

Lightner, D.V. 2012. Biology and Pathology of Early Mortality Syndrome of Shrimp, in: Global Outlook for Aquaculture Leadership. Bangkok, Thailand, p. 40.

Lightner, Redman, R.M.M., Pantoja, C.R.R., Tang, K.F.J.F.J., Noble, B.L.L., Schofield, P., Mohney, L.L.L., Nunan, L.M.M., Navarro, S.A.A., Lightner, D.V. V., Redman, R.M.M., Pantoja, C.R.R., Tang, K.F.J.F.J., Noble, B.L.L., Schofield, P., Mohney, L.L.L., Nunan, L.M.M., Navarro, S.A.A., Lightner, Redman, R.M.M., Pantoja, C.R.R., Tang, K.F.J.F.J., Noble, B.L.L., Schofield, P., Mohney, L.L.L., Nunan, L.M.M., Navarro, S.A.A. 2012. Historic emergence, impact and current status of shrimp pathogens in the Americas. J. Invertebr. Pathol. 110, 174–183. https://doi.org/10.1016/j.jip.2012.03.006

Liu, Y.-M., Qiu, L., Sheng, A.-Z., Wan, X.-Y., Cheng, D.-Y., Huang, J. 2018. Quantitative detection method of *Enterocytozoon hepatopenaei* using *Taq*Man probe real-time PCR. J. Invertebr. Pathol. 151, 191–196. https://doi.org/10.1016/j.jip.2017.12.006

Lo, C., Flegel, T. 2014. Free release of primers for specific detection of bacterial isolates that cause acute hepatopancreatic necrosis disease (AHPND) [WWW Document]. Netw. Aquac. Centres Asia-Pacific, Bangkok, Thailand.Bangkok, Thailand. URL https://enaca.org/?id=88 (accessed 10.24.19).

Mai, H.N., Aranguren Caro, L.F., Cruz-Flores, R., Dhar, A.K. 2021. Development of a Recombinase Polymerase Amplification (RPA) assay for Acute Hepatopancreatic Necrosis Disease (AHPND) detection in Pacific white shrimp (*Penaeus vannamei*). Mol. Cell. Probes 57, 101710. https://doi.org/10.1016/j.mcp.2021.101710

Mai, H.N., Cruz-Flores, R., Aranguren Caro, L.F., White, B.N., Dhar, A.K. 2020a. A comparative study of *Enterocytozoon hepatopenaei* (EHP) challenge methods in *Penaeus vannamei*. J. Invertebr. Pathol. 171, 107336. https://doi.org/10.1016/j.jip.2020.107336

Mai, H.N., Cruz-Flores, R., Dhar, A.K. 2020b. Development of an indirect Enzyme Linked Immunoassay (iELISA) using monoclonal antibodies against *Photorhabdus* insect related toxins, PirA*Vp* and PirB*Vp* released from *Vibrio* spp. J. Microbiol. Methods 176, 106002. https://doi.org/10.1016/j.mimet.2020.106002

Munkongwongsiri, N., Aldama-Cano, D.J., Suebsing, R., Thaiue, D., Prasartset, T., Itsathitphaisarn, O., Sritunyalucksana, K. 2021. Microsporidian *Enterocytozoon hepatopenaei* (EHP) spores are inactivated in 1 min at 75 °C. Aquaculture 533, 736178. https://doi.org/10.1016/j.aquaculture.2020.736178

Nunan, L., Lightner, D., Pantoja, C., Gomez-Jimenez, S. 2014. Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. Dis. Aquat. Organ. 111, 81–86. https://doi.org/10.3354/dao02776

OIE. 2021. JPN 03/04/2021 Alert / Alerte / Alerta - Acute hepatopancreatic necrosis disease / Acute hepatopancreatic necrosis / Necrosis hepatopancreática aguda [WWW Document]. OIE. URL https://mailchi.mp/oie/est-19022021-alertalertealerta-highly-pathogenic-avian-influenzainfluenza-aviaire-hautement-pathogneinfluenza-aviar-altamente-patgena-4729486?e=eb87094465 (accessed 4.19.21).

Park, K.S., Ono, T., Rokuda, M., Jang, M.H., Okada, K., Iida, T., Honda, T. 2004. Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus*. Infect. Immun. 72, 6659–6665. https://doi.org/10.1128/IAI.72.11.6659-6665.2004

Prachumwat, Taengchaiyaphum, Mungkongwongsiri, A., Flegel, Sritunyalucksana. 2019. Update on early mortality syndrome / acute hepatopancreatic necrosis disease by April 2018 5–17. https://doi.org/10.1111/jwas.12559

Rajendran, K. V., Shivam, S., Ezhil Praveena, P., Joseph Sahaya Rajan, J., Sathish Kumar, T., Avunje, S., Jagadeesan, V., Prasad Babu, S.V.A.N.V., Pande, A., Navaneeth Krishnan, A., Alavandi, S. V., Vijayan, K.K. 2016. Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus* (*Litopenaeus*) *vannamei* in India. Aquaculture. https://doi.org/10.1016/j.aquaculture.2015.12.034

Restrepo, L., Bayot, B., Arciniegas, S., Bajaña, L., Betancourt, I., Panchana, F., Reyes Muñoz, A. 2018. Pir*VP* genes causing AHPND identified in a new *Vibrio* species (*Vibrio punensis*) within the commensal Orientalis clade. Sci. Rep. 8, 13080. https://doi.org/10.1038/s41598-018-30903-x

Santos, H.M., Tsai, C., Maquiling, K.R.A., Tayo, L.L., Mariatulqabtiah, A.R., Lee, C., Chuang, K.P. 2019. Diagnosis and potential treatments for acute hepatopancreatic necrosis disease (AHPND): a review. Aquac. Int. 28, 169–185.

Tang, K.F.., Bondad-Reantaso, M.., Arthur, J.R., MacKinnon, B., Hao, B., Alday-Sanz, V., Liang, Y., Dong, X. 2020. Shrimp acute hepatopancreatic necrosis disease strategy manual, FAO Fisheries and Aquaculture Circular NFIA/C1190. Rome-Italy.

Tang, K.F.J., Pantoja, C.R., Redman, R.M., Han, J.E., Tran, L.H., Lightner, D. V. 2015. Development of *in situ* hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. J. Invertebr. Pathol. 130, 37–41. https://doi.org/10.1016/j.jip.2015.06.009

Tangprasittipap, A., Srisala, J., Chouwdee, S., Somboon, M., Chuchird, N., Limsuwan, C., Srisuvan, T., Flegel, T.W., Sritunyalucksana, K. 2013. The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. BMC Vet. Res. 9. https://doi.org/10.1186/1746-6148-9-139

Thornber, K., Verner-Jeffreys, D., Hinchliffe, S., Rahman, M.M., Bass, D., Tyler, C.R. 2019. Evaluating antimicrobial resistance in the global shrimp industry. Rev. Aquac. 1–21. https://doi.org/10.1111/raq.12367

Tinwongger, S., Proespraiwong, P., Thawonsuwan, J., Sriwanayos, P., Kongkumnerd, J., Chaweepack, T., Mavichak, R., Unajak, S., Nozaki, R., Kondo, H., Hirono, I. 2014. Development of PCR diagnosis for shrimp Acute Hepatopancreatic Necrosis Disease (AHPND) strain of *Vibrio parahaemolyticus*. Fish Pathol. 49, 159–164. https://doi.org/10.3147/jsfp.49.159

Tourtip, S., Wongtripop, S., Stentiford, G.D., Bateman, K.S., Sriurairatana, S., Chavadej, J., Sritunyalucksana, K., Withyachumnarnkul, B. 2009. *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): Fine structure and phylogenetic relationships. J. Invertebr. Pathol. https://doi.org/10.1016/j.jip.2009.06.004

Tran, L., Nunan, L., Redman, R.R.M.R., Mohney, L.L.L., Pantoja, C.C.R., Fitzsimmons, K., Lightner, D.V. 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Organ. 105, 45–55. https://doi.org/10.3354/dao02621

Wangman, P., Chaivisuthangkura, P., Sritunyalucksana, K., Taengchaiyaphum, S., Senapin, S., Pengsuk, C., Sithigorngul, P., Longyant, S. 2017. Development of monoclonal antibodies specific to ToxA and ToxB of *Vibrio parahaemolyticus* that cause acute hepatopancreatic necrosis disease (AHPND). Aquaculture 474, 75–81. https://doi.org/10.1016/j.aquaculture.2017.03.039

Research Update on Emergent Shrimp Pathogens in Thailand

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Abstract

Recent evidence suggest that the emergent microsporidian, *Enterocytozoon hepatopenaei* (EHP) is a component cause of white feces syndrome (WFS) in shrimp. The natural WFS shrimp were found to be infected with EHP. At the laboratory level, shrimp induced to be heavily infected with EHP showed no WFS symptom suggesting that the causes of WFS is complex involved with other cause, not only EHP. The other component causes are under investigation. Better understanding of virulence mechanism of EHP infection in shrimp will assist in establishing innovative strategies to reduce its viability and potential infectivity in shrimp farms. Transmission of microsporidia is involved ingestion of spores in the water and the site of initial infection being the gastrointestinal tract. EHP spore is having a thick, protective chitinous wall around the cell membrane that allows them to survive outside their hosts and involve with the microsporidian pathogenesis. Here we describe successful purification of active EHP spores with a novel spore viability assay based on polar-tube extrusion or germination triggered by Phloxin B. The physical conditions such as temperature and PH, and chemical factors such as KMnO4, and chlorine that affect spore germination were examined as a practical guideline for the inactivation of the spores at a farm level. The potential environmental reservoir of EHP were found to be a mussel of the genus *Mytilopsis*, which is found frequently in the water canal or pipe in the shrimp rearing system. Recent evidence demonstrates that the mussel can be infected by EHP and can transmit EHP to shrimp in the laboratory model.

Keywords: White feces syndrome (WFS), Enterocytozoon hepatopenaei (EHP), shrimp slow growth, environmental reservoir, Mytilopsis

Establishment of Threshold Infection Levels of WSSV in Different Weight Ranges of *Penaeus vannamei* Using Quantitative PCR (qPCR)

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Abstract

Threshold infection level is the pathogen load of the test animals measured before the appearance of clinical signs and mortality. This study aims to establish the threshold infection levels of WSSV in different weight ranges of *Penaeus vannamei* using qPCR. Artificial infection experiments were conducted using four weight ranges (3–5 g, 7–8 g, 15–18 g, and 22–25 g). The LD_{50} of the different weight ranges of shrimps were achieved at viral dilution of 10^{-6} and 10^{-5} after 216–240 hpi, and the viral loads of these inoculums have a range of 10^5–10^6 WSSV DNA copies/g. The viral loads of the samples in the time-course infection experiments when the mortalities started was determined at 10^9 WSSV DNA copies/g, while for the survivors was at 10^6 WSSV DNA copies/g. The threshold infection level of WSSV in shrimp was determined at 10^7 to 10^8 WSSV DNA copies/g. It was also found out that the threshold infection level was not weight dependent.

Keywords: Threshold infection levels, White Spot Syndrome Virus, Penaeus vannamei, Quantitative PCR

Introduction

Viral diseases have caused major constraints in shrimp farming in most Asian countries and the world. According to the OIE List of Crustacean Diseases for 2020, White Spot Syndrome Virus (WSSV) is still included since its first emergence and outbreaks in the early 1990s from China, Japan, Taiwan, and northern Thailand, and was subsequently spread into southern Thailand, India, and Indonesia (Lotz, 1997). Experimental evidence suggests that WSSV can be transferred horizontally and vertically through water, carrier organism, cannibalism, or infected broodstock (Flegel, 1997; de la Peña *et al*., 2007; Hoa *et al*., 2011). Approximately 3–10 days following onset of infection, mortality rates typically reach levels greater than 80% and usually 100 % of the population (Nakano *et al*., 1994; Kasornchandra *et al*., 1996; Lightner, 1996).

WSSV was first detected in the Philippines in early 1999 using PCR and Western blot assays in different life stages of cultured *Penaeus monodon*. Moreover, out of the

71 samples analyzed, there were 51 (72 %) were WSSV-positive (both for 1-step and 2-step, non-nested PCR) (Magbanua *et al*., 2000). Prevalence of WSSV in wild-caught *P. monodon* was also reported in the different sampling sites in the Philippines (de la Peña *et al*., 2007). Collection of these contaminated, wild *P. monodon* as spawners or broodstocks could serve as the primary source of WSSV contamination in shrimp farms due to vertical transmission of the virus in hatcheries (de la Peña *et al*., 2007).

The continued outbreaks of viral diseases necessitate the aquaculture industry to establish preventive and control measures to mitigate its negative impacts in production both in the hatchery and grow-out phases. There have been several experimental runs on developing novel vaccines and chemotherapeutants to date. However, there were no consistently effective methods (OIE, 2019). The majority of the shrimp farms adopted the domestication of specific pathogen-free (SPF) shrimp stocks. Other prevention and control methods provide immunostimulants and probiotics in the grow-out phase and practice disinfection of broodstock or spawners and eggs. Another emerging approach is breeding specific pathogen-resistant (SPR) shrimp stocks (Cuellar-Anjel *et al*., 2012; Huang *et al*., 2011).

On the other hand, pro-active monitoring and early detection of devastating pathogens are the most efficient responses so that immediate and appropriate interventions to prevent and control the spread of infection can be implemented. The main objective of this study is to determine the threshold infection levels of WSSV in different weight ranges of *P. vannamei* using qPCR and to know if it is weight dependent. The established threshold infection levels will enable the farmers to strictly monitor the health status of the cultured shrimps, and it will also serve as a reference for the ideal viral load to maintain in the farm to avoid disease outbreaks.

Materials and methods

Screening and maintenance of experimental animals

Experimental animals (*P. vannamei*) were obtained from a shrimp grow-out farm in Zarraga, Iloilo. The weight ranges needed for the infection experiments were 3–5 g, 7–8 g, 15–18 g, and 22–25 g. Before the procurement and stocking, the experimental animals were screened for WSSV and AHPND using conventional PCR. If the results were negative, the experimental animals were stocked in 250 L fiberglass tanks and maintained in a flow-through seawater system at salinity of 29–32 ppt, water temperature of 25–30 °C, and constant supply of aeration and UV-sterilized seawater at the Infection Building, Wet Laboratory Complex, SEAFDEC/AQD. Commercial shrimp feed was given twice a day (9:00 AM and 3:00 PM) to the shrimp stocks with feeding rate of 3 % body weight.

Inoculum preparation and tissue passage

The inoculum was prepared by homogenizing WSSV one-step positive shrimp tissues (de la Peña *et al*., 2015). Approximately 0.75 g of muscle tissue was homogenized with 6.75 ml of phosphate-buffered saline (PBS) and centrifuged at 9,000 rpm for 10 min at 4 °C. The supernatant was then filtered with a 0.45 µm pore size syringe filter. The supernatant containing the virus was diluted with the ratio of 1:10 parts (filtrate: PBS; 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}). The dilution used for the tissue passage was 10^{-4}.

Ten aquaria were filled with 10 L of UV-sterilized seawater with salinity of 30 ppt, supplied with constant aeration, and the water temperature was maintained at 25 °C. In each aquarium, ten shrimps (ABW 18 g) were stocked and acclimatized for 8 hours (h). The positive control groups were intramuscularly injected with 100 µl of the viral inoculum while 100 µl of PBS was given to the negative control. Mortalities were monitored and recorded every 6 h. The dead shrimps were collected, dissected into different sections (head, body, and tail), placed and labeled in an individual resealable plastic bag, and stored at -80 °C biofreezer. Two tissue passages were conducted to increase the virulence of the viral isolate and the volume of infected tissues. Conventional PCR analysis was conducted to confirm that the shrimps were successfully infected with WSSV. The shrimp samples that were 1-step PCR positive for WSSV were used as a source of viral inoculum for the following artificial infection experiments.

DNA extraction

Total genomic DNA was extracted from the gill tissue of the infected shrimps using DNAzol Reagent (MRC, USA) and following the manufacturer's protocol. Briefly, approximately 50 mg of gill tissue were homogenized in 1 ml DNAzol, followed by centrifugation for 10 min at 14,800 x g at 4 °C and transfer of the supernatant to a new tube. DNA was precipitated by the addition of 0.5 ml of 100 % ethanol. Pelleted DNA was washed twice with 1 ml of 75 % ethanol by centrifugation and air-dried for a few seconds. The dried DNA pellets were suspended in 100 µl of 8 mM NaOH, incubated at 45 °C for 15 min, after which 10 µl of TE buffer was added for storage at -20 °C. The concentration and purity of the extracted DNA were measured using a nano-spectrophotometer (IMPLEN, Germany).

Detection of WSSV using conventional PCR

The infected shrimp samples were submitted to l-step and nested PCR tests using the WSSV -specific primer pairs designed by Kimura *et al.* (1996). PCR reactions were carried out in a 25 µl reaction mixture. Amplification was performed in a programmable thermal cycler (Eppendorf, Germany) with the following cycle parameters: the initial heating at 72 °C for 10 min and 95 °C for 6 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 1 min with a final extension at 72 °C for 5 min before holding at 4 °C until ready for electrophoresis. For the nested PCR step, 1.0 µl of the post-1-step PCR was used as the template for PCR amplification using the primer pair P3-P4 with the protocol described above.

The PCR products were separated in 2 % agarose gel, stained with Gel Red Nucleic Acid Stain (Biotium, USA), and visualized using the DigiDoc-It® Imaging System (Analytik Jena, USA). The one-step and nested primer pairs amplified products were 982 bp and 570 bp, respectively.

Determination of viral load using qPCR

Rotor-Gene RG3000 (Corbett Research, Australia) real-time PCR machine and fluorescent dye KAPA SYBR® FAST qPCR Master Mix Kits (KAPA Biosystems, USA) were used for the real-time PCR analysis of the WSSV viral load. The amplification profile was performed and programmed

in the Rotor-Gene RG3000 with the following cycle parameters: the initial heating at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 1 min and extension at 72 °C for 1 min with a final extension at 72 °C for 5 min. Real-time PCR was conducted in a 13 µl reaction using 1 µl of the original viral inoculum (unfiltered, undiluted preparation), 6.25 µl of SYBR Green Master Mix, 0.25 µl of each P1-P2 primer (Kimura *et al.* 1996; 10 mM final concentration), and 4.75 µl of ultrapure distilled water (Invitrogen, USA). In each of the 36-wells real-time PCR run, a dilution series of the plasmid standard of WSSV was run along with the unknown samples and no template controls (NTC).

Determination of LD_{50}

Preliminary infection experiments using exponential serial dilutions were performed to determine the range of inoculum for LD_{50}. The viral load of the prepared inoculum was determined using qPCR before the preliminary infection experiment. Six dilutions (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10}) of the viral inoculum were prepared from WSSV infected shrimp tissue.

Individual LD_{50} was conducted to four weight ranges. The experimental set-ups consist of 10 aquaria filled with 10 L of UV-sterilized seawater with salinity of 30 ppt, supplied with constant aeration, and water temperature maintained at 25 °C. In each aquarium, ten shrimps were stocked and acclimatized for 8 h before the injection of the viral inoculum. The experimental groups were injected intramuscularly with 100 µl of the viral inoculum, and the negative control groups received 100 µl of PBS. The shrimps were monitored and observed for mortalities every 6 h. The dead shrimps were collected, dissected into different sections (head, body, and tail), and stored at -80 °C. The rearing water was changed every day by 50 % through siphoning.

Time-course experiment

Time-course experiments were conducted on all four different weight ranges after the appropriate viral dilution was determined during the LD_{50}. The experimental set-ups consist of 10 aquaria filled with 10 L of UV-sterilized seawater, salinity of 30 ppt, supplied with constant aeration, and water temperature maintained at 25 °C. In each aquarium, ten shrimps were stocked and acclimatized for 8 h before injection of the viral inoculum. The experimental groups were intramuscularly injected with 100 µl of the 10^{-6} dilution of the viral inoculum, and the negative control received only 100 µl of PBS. The proper time interval of sampling was followed in order to monitor the increase of the viral load. Every sampling time, 2 shrimps were collected, dissected into different sections (head, body, and tail), and stored at -80 °C biofreezer prior to the qPCR test. The rearing water was changed every day by 50 % through siphoning.

Results

Screening of experimental animals

Several batches of experimental animals were all nested PCR negative for WSSV and AHPND, as shown in **Figures 1** and **2**.

Tissue passage

To increase the virulence of WSSV, at least two tissue passages were conducted. The first batch of 35 shrimps (ABW 18 g) were all one-step PCR positive. For the second

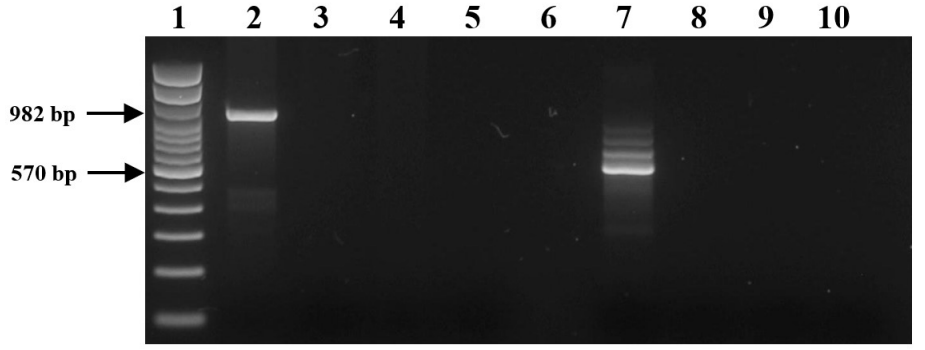

Figure 1. Agarose gel electrophoresis of the products from WSSV 1-step and nested PCR amplifications using specific primers designed by Kimura et al. (1996). Lanes (1) 100 bp DNA marker, (2) 1-step positive control, (3-4) experimental shrimps, (5) negative control, (6) empty, (7) nested positive control, (8-9) experimental shrimps, and (10) negative control

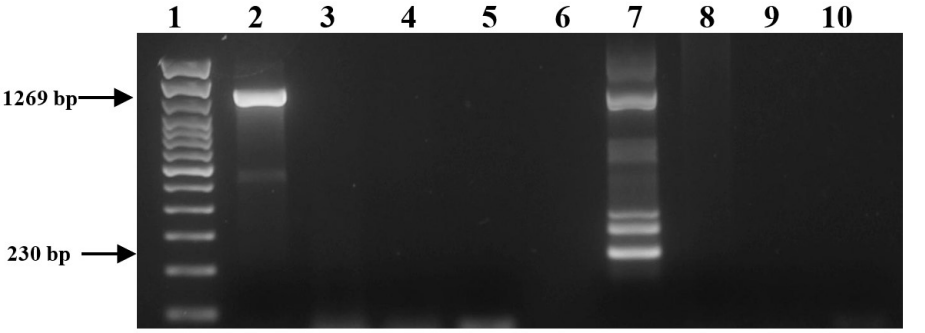

Figure 2. Agarose gel electrophoresis of the products from AHPND 1-step and nested PCR amplifications using specific primers designed by Dangtip et al. (2015). Lanes (1) 100 bp DNA marker, (2) 1-step positive control, (3-4) experimental shrimps, (5) negative control, (6) empty, (7) nested positive control, (8-9) experimental shrimps, and (10) negative control

batch of 40 shrimps (ABW 18 g), 30 out of 40 were found to be one-step PCR positive. Viral loads of infected shrimp from the tissue passages were determined using qPCR. The viral loads of 1-step PCR positive shrimps ranged from 2.7 x 10^9 to 5.1 x 10^9 WSSV DNA copies/g, while the nested PCR positive shrimps ranged from 6.4 x 10^6 to 8.9 x 10^6 WSSV DNA copies/g.

Establishment of the standard curve for qPCR

The optimization and establishment of the qPCR protocol were earlier conducted under the Government of Japan-Trust Fund 5 (GOJ-TF 5) Project. WSSV-infected muscle tissues and WSSV-purified plasmid were used in the establishment of the standard curve. The plasmid concentration was estimated to be 4.40 x 10^{10} WSSV DNA copies/µl. Serial 4-fold dilutions of the plasmid were used for the standard curve to quantify WSSV genomic DNA. As shown in **Figure 3**, the standard curve has an R^2 equal to 0.98466. The real-time PCR protocol has a detection limit of 33 DNA copies/µl (unpublished results).

Determination of LD_{50}

Six dilutions (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10}) of the viral inoculum were prepared and used for the preliminary infection experiments. Summary of the viral inoculum dilution and number of days for the LD_{50} were shown in **Table 1**. For weight ranges 3–5 g and 7–8 g, the LD_{50} was determined at the dilution of 10^{-6} and was achieved after 216 and 240 hours post-infection (hpi), respectively. While for weight ranges 15–18 g and 22–25 g, the LD_{50} was at the dilution of 10^{-5} and was achieved after 216 and 240 hpi, respectively. The viral load of the 10^{-6} dilution of inoculum was 7.7 x 10^5 DNA copies/g, while for 10^{-5} was 6.1 x 10^6 DNA copies/g.

Determination of the threshold infection levels

Summary of the average viral loads of each sampling point for all the time-course infection experiments were presented in **Table 4**. The mortalities of weight range 3–5 g have started at 195 hpi with a viral load ranging from 1.6 x 10^9 to 3.3 x 10^9 WSSV DNA copies/g. While the range of the viral load of the survivors were from 3.3 x 10^6 to 4.1 x 10^6 WSSV DNA copies/g after 231 hpi. The mortalities of weight range 7–8 g have started at 231 hpi with a viral load ranging from 3.7 x 10^9 to 5.1 x 10^9 WSSV DNA copies/g. While the range of the viral load of the survivors were from 6.3 x 10^6 to 8.9 x 10^6 WSSV DNA copies/g after 264 hpi. The mortalities of weight range 15-18 g have started at 162 hpi with a viral load ranging from 1.2 x 10^9 to 4.6 x 10^9 WSSV DNA copies/g. While the range of the viral load of the survivors were from 6.8 x 10^6 to 8.8 x 10^6 WSSV DNA copies/g after 219 hpi. The mortalities of weight range 22–25 g have started at 237 hpi with a viral load ranging from 1.4 x 10^9 to 5.1 x 10^9 WSSV DNA copies/g. While the range of the viral load of the survivors were from 5.1 x 10^6 to 9.3 x 10^6 WSSV DNA copies/g after 255 hpi.

The mortalities, survival, and manifestation of clinical signs were carefully correlated in determining the threshold level of WSSV in shrimp. As shown in **Table 4**, there were late onsets on the increase of viral loads observed for weight ranges 3–5 g and 7–8 g, which took about 183–219 hpi to increase from 10^6 to 10^7 WSSV DNA copies/g. On the contrary, for weight ranges, 15–18 g and 22–25 g, early onsets of viral load increase were observed around 24–48 hpi to increase from 10^6 to 10^7 WSSV DNA copies/g. As the viral load reached 109 WSSV DNA copies/g, clinical signs and mortalities were observed.

Discussion

From the first emergence of WSSV until the early 2000s, the approximate economic loss of Asian countries was suggested to reach US$ 1 Billion per annum (Briggs *et al.*,

Table 1. Viral inoculum dilutions and time to reach LD_{50} in 4 weight ranges

Weight ranges	LD_{50} Viral dilution	Hours post-infection (hpi)
3-5	10^{-6}	216 hpi
7-8	10^{-6}	240 hpi
15-18	10^{-5}	216 hpi
22-25	10^{-5}	240 hpi

Table 2. Viral loads of 4 weight ranges during the time-course infection experiments that mortalities have started

Weight ranges	LD_{50} Viral dilution	Average
3-5	1.60×10^9 - 3.30×10^9	2.58×10^9
7-8	3.70×10^9 - 5.10×10^9	4.48×10^9
15-18	1.20×10^9 - 4.60×10^9	3.36×10^9
22-25	1.40×10^9 - 5.10×10^9	3.81×10^9

Table 3. Viral loads of the survivors of 4 weight ranges during the time-course infection experiments

Weight ranges	LD_{50} Viral dilution	Average
3-5	3.30×10^6 - 4.10×10^6	23.86×10^6
7-8	6.30×10^6 - 8.90×10^6	7.62×10^6
15-18	6.80×10^6 - 8.80×10^6	7.90×10^6
22-25	5.10×10^6 - 9.30×10^6	7.48×10^6

Table 4. Summary of the viral loads of the samples of 4 weight ranges during the time course infection experiments

3-5 g		7-8 g		15-18 g		22-25g	
hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g
0	3.79×10^6	0	3.71×10^6	0	3.78×10^6	0	3.71×10^6
24	4.03×10^6	24	4.36×10^6	24	5.64×10^7	24	6.75×10^6
48	3.93×10^6	48	3.90×10^6	48	2.34×10^7	48	1.05×10^7
72	3.52×10^6	72	5.00×10^6	72	8.04×10^7	72	1.77×10^7
96	3.00×10^6	96	4.70×10^6	96	2.31×10^8	96	2.85×10^7
120	3.74×10^6	120	6.04×10^6	120	4.72×10^8	120	4.07×10^7
132	4.10×10^6	144	5.90×10^6	132	3.82×10^8	144	3.90×10^7
144	4.24×10^6	156	6.50×10^6	144	7.88×10^8	156	7.40×10^7
156	3.93×10^6	168	7.00×10^6	156	9.89×10^8	168	8.47×10^7
162	4.00×10^6	180	6.90×10^6	162	1.28×10^9	180	7.00×10^7
168	3.23×10^6	192	8.15×10^6	168	2.01×10^9	192	9.98×10^7
174	4.15×10^6	198	7.74×10^6	174	1.90×10^9	198	8.97×10^7
180	3.50×10^6	204	9.01×10^6	180	3.92×10^9	204	8.96×10^7
183	1.04×10^7	210	8.80×10^6	183	2.89×10^9	210	9.91×10^7

3-5 g		7-8 g		15-18 g		22-25g	
hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g	hpi	WSSV DNA copies/g
186	5.05×10^{7}	213	9.99×10^{6}	186	2.10×10^{9}	213	8.45×10^{7}
189	2.01×10^{8}	216	8.48×10^{6}	189	3.60×10^{9}	216	7.34×10^{7}
192	7.30×10^{8}	219	7.86×10^{7}	192	4.00×10^{9}	219	9.86×10^{7}
195	1.96×10^{9}	222	1.46×10^{8}	195	4.92×10^{9}	222	9.85×10^{7}
198	2.01×10^{9}	225	1.94×10^{8}	198	3.83×10^{9}	225	7.85×10^{7}
201	3.02×10^{9}	228	9.47×10^{8}	201	2.90×10^{9}	228	9.56×10^{7}
204	1.98×10^{9}	231	4.61×10^{9}	204	4.10×10^{9}	231	6.58×10^{7}
207	2.79×10^{9}	234	3.71×10^{9}	207	4.59×10^{9}	234	9.41×10^{7}
210	1.68×10^{9}	237	4.04×10^{9}	210	3.79×10^{9}	237	1.40×10^{9}
213	3.03×10^{9}	240	3.99×10^{9}	213	4.19×10^{9}	240	2.97×10^{9}
216	3.00×10^{9}	243	4.57×10^{9}	216	3.75×10^{9}	243	3.73×10^{9}
219	2.94×10^{9}	246	4.54×10^{9}	219	6.84×10^{6}	246	5.15×10^{9}
225	3.37×10^{9}	249	4.19×10^{9}	225	$8,.82 \times 10^{6}$	249	5.00×10^{9}
231	3.49×10^{6}	252	5.05×10^{9}	231	7.45×10^{6}	252	4.60×10^{9}
237	3.93×10^{6}	255	4.93×10^{9}	237	8.43×10^{6}	255	5.13×10^{6}
243	4.14×10^{6}	258	4.57×10^{9}	243	7.42×10^{6}	258	5.89×10^{6}
243	4.00×10^{6}	261	5.12×10^{9}	243	7.20×10^{6}	261	7.94×10^{6}
255	3.83×10^{6}	264	7.04×10^{6}	255	7.42×10^{6}	264	7.30×10^{6}
279	3.59×10^{6}	267	8.39×10^{6}	279	8.01×10^{6}	267	6.73×10^{6}
303	4.22×10^{6}	270	6.31×10^{6}	303	8.74×10^{6}	270	5.76×10^{6}
327	3.65×10^{6}	276	7.80×10^{6}	327	8.64×10^{6}	276	9.36×10^{6}
		282	6.34×10^{6}			282	7.04×10^{6}
		288	8.91×10^{6}			288	8.50×10^{6}
		294	8.12×10^{6}			294	7.59×10^{6}
		306	7.84×10^{6}			306	9.31×10^{6}
		318	7.77×10^{6}			318	9.15×10^{6}

2005). This massive economic loss can be attributed to high mortality rates that can reach up to 100 % within 10 days (Flegel, 1997). Even with the advancement of shrimp aquaculture systems, like the semi-closed, use of pond liners, shrimp "toilet", and biofloc, disease outbreaks caused by WSSV is still considered as the number one threat in the sustainability of the industry worldwide.

In this study, the LD_{50} of the different weight ranges (3–5 g, 7–8 g, 15–18 g, and 22–25 g) of shrimps were achieved at viral

dilution of 10^{-6} and 10^{-5} after 216–240 hpi. The viral loads of the inoculum have a range of 10^5–10^6 WSSV DNA copies/g. These viral loads were parallel to the results of Durand and Lightner (2002), Meng *et al.* (2010), and Jeswin *et al.* (2013) that the presence of 10^5 to 10^7 WSSV DNA copies/g is sufficient to infect shrimps through immersion or intramuscular injection. The LD_{50} results were used to determine the appropriate viral inoculum to be injected and the sampling frequencies of the time-course infection experiments.

The average viral loads present in the samples when the mortalities have started in all the time-course infection experiments were 2.58 x 10^9, 4.48 x 10^9, 3.36 x 10^9, and 3.81 x 10^9 WSSV DNA copies/g, while for the surviving shrimps were 3.86 x 10^6, 7.62 x 10^6, 7.90 x 10 10^6, and 7.48 x 106 WSSV DNA copies/g, for weight ranges 3–5 g, 7–8 g, 15–18 g, and 22–25 g, respectively. Tang and Lightner (2000) were able to classify the infection level of WSSV in juvenile shrimps (1 g) based on the relationship between the viral load of the samples and the severity of the infection. According to their classification, the average viral loads of the samples from all the time-course infection experiments when the mortalities occurred were under moderate to severe (G3, 2 x 10^9 WSSV DNA copies/g) classification, while the survivors were classified as mild (G1, 2 x 10^5 WSSV copies/g). Based on their observations, samples with infection level G3 became moribund after 35–60 hpi. Our observations for weight ranges 3–5 g, 7–8 g, 15–18 g, and 22–25 g the mortalities started at 195 hpi, 231 hpi, 162 hpi, and 237 hpi, respectively. Furthermore, the viral loads of other penaeid shrimps that were subjected to artificial infection experiments which resulted in mortalities were quantified: in artificially infected juvenile *P. vannamei* (7.50 x 10^8 to 2.5 x 10^9 copies/µg of total DNA) and juvenile *Feneropenaeus chinensis* (5.72 x 10^5 to 9.6 x 10^5 copies/ng of DNA) (Durand and Lightner, 2002; Sun *et al.*, 2012).

According to Sun *et al.* (2012), the turning point of chronic infection to acute infection in juvenile *F. chinensis* (4–8 g) was suggested from 27 to 30 hpi when the viral load increased from 10^3 to 10^4 copies/ng of DNA. Moreover, the substantial increase of the viral load to 10^5 copies/ng of DNA explained the symptoms of skin color changes, reduced food consumption, and gathering at the water surface due to dyspnea, resulting in mortality. In our time-course experiments, we observed that the viral loads of the lighter weight ranges 3–5 g and 7–8 g increased by one exponential order (from 10^6 to 10^7 WSSV DNA copies/g) after 180 to 216 hpi, while the heavier weight ranges 15–18 g and 22–25 g were earlier after 24 to 48 hpi. However, no clinical signs and mortalities were observed in this viral load range (10^6 to 10^7 WSSV DNA copies/g). It was only when the viral load reached up to 10^9 WSSV DNA copies/g that clinical signs and mortalities were observed.

Viral loads of WSSV positive shrimp submitted to Fish Health Section Diagnostic Laboratory, SEAFDEC/AQD were also determined. This was done to compare the viral loads of artificially and naturally infected shrimp samples. The WSSV positive samples were divided into two groups, 1-step and nested. The viral loads of naturally infected 1-step and nested PCR positive samples ranged from 3.2 x 10^9 to 5.1 x 10^9 WSSV DNA copies/g and 7.4 x 10^3 to 1.2 x 10^t WSSV DNA copies/g, respectively. Mendoza-Cano and Sanchez-Paz (2013) were able to determine the viral load of WSSV in wild samples of marine crustaceans: *Macrobrachium rosenbergii* (3.4 x 10^7 DNA copies/ µl), *Penaeus vannamei* (1.28 x 10^6 DNA copies/ µl), *P. stylirostris* (2.13 x 10^4

DNA copies/µl), *Callinectes bellicosus* (5.98 x 10^3 DNA copies/µl) and *Calanus pacificus californicus* (6.1 x 10^5 DNA copies/µl). Also, our results conformed to the quantification of naturally infected cultured penaeid shrimps (2.1 x 10^8 to 2.64 x 10^{14} copies/g of shrimp tissue) (Siddique *et al*., 2018).

The time-course infection experiment was designed based on the results of LD_{50} in different weight ranges of shrimp. The viral inoculum used was 10^{-6} with a viral load of 10^5 WSSV DNA copies/g. Also, we quantified and correlated the viral loads of the naturally and artificially infected shrimps. The viral load of naturally infected nested PCR positive samples (10^3–10^5 DNA copies/g) was less than compared to the viral inoculum used in the LD50, while the 1-step PCR positive samples (10^9 WSSV DNA copies/g) were more than compared to the established threshold infection level that resulted in mortalities in artificially infected shrimps. Based on the viral loads of artificially and naturally infected shrimps, we elucidated that the threshold infection levels of WSSV in *P. vannamei* was from 10^7 to 10^8 WSSV DNA copies/g. In this viral load range, there were no observable clinical signs and mortalities. Also, we have observed that in all weight ranges, the threshold infection levels are the same. Therefore, it is not weight dependent.

To date, there is no available data on the threshold infection levels of WSSV in penaeid shrimps. Hence, in this study, we established the threshold infection levels of WSSV in different weight ranges of *P. vannamei* from 10^7 to 10^8 WSSV DNA copies/g. These established threshold infection levels can be used as a reference by shrimp farmers and laboratory analysts to facilitate stringent and proactive health monitoring of the shrimp stocks on the farm. Also, it will give the farmers adequate time to implement appropriate interventions before the viral load reaches the threshold infection levels that will result in WSSV outbreak.

Acknowledgment

We are very grateful to the Government of Japan-Trust Fund (study code: FH-07-F2015T) and SEAFDEC/AQD for funding this work. We thank Mr. Ivan Yaptangco of IJY Shrimp Farm for providing the experimental animals and the staff of Fish Health Section Diagnostic Services for the technical support.

References

Briggs, M., S. Funge-Smith, R.P. Subasinghe and M. Phillips. 2005. Introductions and movement of two penaeid shrimp species in Asia and the Pacific. FAO Fisheries Technical Paper. No. 476. FAO, Rome. 78 pp

Cuéllar-Anjel, J., White-Noble, B., Schofield, P., Chamorro, R., & Lightner, D. V. 2012. Report of significant WSSV-resistance in the Pacific white shrimp, *Litopenaeus vannamei*, from a Panamanian breeding program. Aquaculture, 368–369, 36–39. https://doi.org/10.1016/j.aquaculture.2012.08.048

de la Peña, D., Lavilla-Pitogo, C., Villar, C., Paner, M., Sombito, C., & Capulos, G. 2007. Prevalence of white spot syndrome virus (WSSV) in wild shrimp *Penaeus monodon* in the Philippines. Diseases of Aquatic Organisms, 77, 175–179. https://doi.org/10.3354/dao01834

de la Peña, L., Cabillon, N., Catedral, D., Amar, E., Usero, R., Monotilla, W., Calpe, A., Fernandez, D., & Saloma, C. 2015. Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. Diseases of Aquatic Organisms, 116(3), 251–254. https://doi.org/10.3354/dao02919

Durand, S. V., & Lightner, D. V. 2002. Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. Journal of Fish Diseases, 25(7), 381–389. https://doi.org/10.1046/j.1365-2761.2002.00367.x

Fish Vet Group Asia Limited, Muang Chonburi, Chonburi, Thailand, & Shinn, A. P. 2018. Asian Shrimp Production and the Economic Costs of Disease. Asian Fisheries Science, 31S. https://doi.org/10.33997/j.afs.2018.31.S1.003

Flegel, T. W. 1997. Special topic review: Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. 13, 10.

Hoa, T. T. T., Zwart, M. P., Phuong, N. T., Vlak, J. M., & de Jong, M. C. M. 2011. Transmission of white spot syndrome virus in improved-extensive and semi-intensive shrimp production systems: A molecular epidemiology study. Aquaculture, 313(1–4), 7–14. https://doi.org/10.1016/j.aquaculture.2011.01.013

Huang, Y.-C., Yin, Z.-X., Ai, H.-S., Huang, X.-D., Li, S.-D., Weng, S.-P., & He, J.-G. 2011. Characterization of WSSV resistance in selected families of *Litopenaeus vannamei*. Aquaculture, 311(1–4), 54–60. https://doi.org/10.1016/j.aquaculture.2010.11.032

International Office of Epizootics. (2019). Aquatic animal health code.

Jeswin, J., Anju, A., Thomas, P. C., Paulton, M. P., & Vijayan, K. K. 2015. Analysis of viral load between different tissues and rate of progression of white spot syndrome virus (WSSV) in *Penaeus monodon*. Aquaculture Research, 46(8), 2003–2012. https://doi.org/10.1111/are.12357

Kasornchandra, J., Boonyaratpalin, B., Khongpradit, R., & Akpanithanpong, U. 1995. Mass mortality caused by systemic bacilliform virus in cultured Penaeid shrimp, *Penaeus monodon*. *Thailand. Asian Shrimp News*, 5(2), 2-3.

Kimura T., Yamano K., Nakano H., Momoyama K., Hiraoka M., & Inouye K. 1996. Detection of Penaeid Rod-shaped DNA Virus(PRDV) by PCR. Fish Pathology, 31(2), 93–98. https://doi.org/10.3147/jsfp.31.93

Lotz, J. M. 1997. Special topic review: Viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. 13, 9.

Lightner, D. V. 1996. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp.

Magbanua, F., Natividad, K., Migo, V., Alfafara, C., de la Peña, F., Miranda, R., Albaladejo, J., Nadala, E., Loh, P., & Mahilum-Tapay, L. 2000. White spot syndrome virus (WSSV) in cultured *Penaeus monodon* in the Philippines. Diseases of Aquatic Organisms, 42, 77–82. https://doi.org/10.3354/dao042077

Mendoza-Cano, F., & Sánchez-Paz, A. 2013. Development and validation of a quantitative real-time polymerase chain assay for universal detection of the White Spot Syndrome Virus in marine crustaceans. Virology Journal, 10(1), 186. https://doi.org/10.1186/1743-422X-10-186

Meng, X.-H., Jang, I. K., Seo, H.-C., & Cho, Y.-R. 2010. A TaqMan real-time PCR assay for survey of white spot syndrome virus (WSSV) infections in *Litopenaeus vannamei* postlarvae and shrimp of farms in different grow-out seasons. Aquaculture, 310(1–2), 32–37. https://doi.org/10.1016/j.aquaculture.2010.10.010

Siddique, M. A., Haque, Md. I.-M., Sanyal, S. K., Hossain, A., Nandi, S. P., Alam, A. S. M. R. U., Sultana, M., Hasan, M., & Hossain, M. A. 2018. Circulatory white spot syndrome virus in South-West region of Bangladesh from 2014 to 2017: Molecular characterization and genetic variation. AMB Express, 8(1), 25. https://doi.org/10.1186/s13568-018-0553-z

Sun, Y., Li, F., & Xiang, J. 2013. Analysis on the dynamic changes of the amount of WSSV in Chinese shrimp *Fenneropenaeus chinensis* during infection. Aquaculture, 376–379, 124–132. https://doi.org/10.1016/j.aquaculture.2012.11.014

Tang, K. F. J., & Lightner, D. V. 2000. Quantification of white spot syndrome virus DNA through a competitive polymerase chain reaction. Aquaculture, 189(1–2), 11–21. https://doi.org/10.1016/S0044-8486(00)00367-7

Efficacy of the Inactivated Nervous Necrosis Virus Vaccine Against Viral Nervous Necrosis in Pond-Reared Orange-Spotted Grouper *Epinephelus coioides*

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Abstract

The field efficacy of the formalin-inactivated nervous necrosis virus (NNV) against viral nervous necrosis (VNN) in orange-spotted grouper (*Epinephelus coioides*) reared in floating net cages in earthen pond was investigated. Seroneutralization assay conducted on the sera of vaccinated fish exhibited the occurrence of neutralizing antibody titers from Day 30 (mean titer 1:1792±701) to Day 150 (1:704±351) with the highest titer observed at Day 60 (1:6656±3435) post-vaccination. Because mortality attributed to VNN was not encountered during the pond experiment, intramuscular challenges of vaccinated and unvaccinated (L-15 injected) fish with NNV ($10^{6.5}$ $TCID_{50}$/fish) were conducted in indoor tanks at Day 30 (Mean body weight [MBW]: vaccinated [21±3.4 g]; unvaccinated [20.6±1 g) and Day 120 (MBW: vaccinated [178±27 g]; unvaccinated [176±19 g) post-vaccination, respectively, to demonstrate the *in vivo* efficacy of the inactivated vaccine. Nil and 25 % mortality rate were obtained in vaccinated and control fish, respectively, challenged with NNV at Day 30 post-vaccination. On the contrary, nil mortality were obtained in both groups challenged with NNV at Day 120 post-vaccination. Although nil mortality was obtained in NNV-challenged unvaccinated fish, 30 % of the fish manifested dark coloration of the skin and abnormal swimming behavior that commenced and disappeared at Day 3 and Day 7 post-NNV challenge, respectively, suggesting an age/weight-dependent resistance to the disease. Our current data illustrate that single vaccination with inactivated vaccine could mount the production of protective antibodies and concomitant conferment of protection against VNN in groupers especially during the early phase of grow-out culture in earthen ponds where they are highly susceptible to the disease.

Key words: Inactivated vaccine, viral nervous necrosis, VNN, Epinephelus coioides

Introduction

Viral nervous necrosis (VNN) is a destructive disease of both farmed and wild fish, with more than 120 species belonging to 30 families from 11 different orders being susceptible to the disease (Bandín and Souto, 2020). Betanodaviruses (family *Nodaviridae*), the causal agents of VNN, are small, non-enveloped, spherical (25–30 nm) viruses with a genome composed of two single-stranded RNA segments: RNA1 (3.1 kb) and RNA 2 (1.4 kb) which encode the viral replicase (110 kDa) and the coat

protein (42 kDa), respectively (Comps *et al*., 1994; Mori *et al*., 1992; Thiery *et al*., 2012). A third RNA segment, RNA3 (0.4 kb), is sub-genomically transcribed from RNA1 in the infected cells and correspondingly encode a protein with potent RNA silencing-suppression activity (Iwamoto *et al*., 2005; Sommerset and Nerland, 2004). Four genotypes have been designated based on the coat protein gene sequences including striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), redspotted grouper nervous necrosis virus (RGNNV), and barfin flounder nervous necrosis virus (BFNNV) (Nishizawa *et al*., 1997). Among these, RGNNV and genetically related viruses have by far been implicated in mass mortalities of hatchery-reared high-value marine fish species including groupers (*Epinephelus* spp.), sea bass (*Lates calcarifer*), and pompano (*Trachinotus blochii*) in the Philippines (Maeno *et al*., 2004, 2002; Pakingking *et al*., 2011).

Control of VNN via elimination of virus-carrying broodfish by RT-PCR (Nishizawa *et al*., 1994) and disinfection of eggs by ozonation (Mori *et al*., 1998) has been carried out but despite the rigid screening of broodstocks for nervous necrosis virus (NNV) by RT-PCR and cell culture isolation, sporadic outbreaks of VNN still occur. Because of the ubiquity of NNV in the marine environment, unpredictable outbreaks of VNN in floating net cages in the open sea is also inevitable (Nakai *et al*., 2009). In addition, broodstocks obtained from the wild, though apparently healthy, most often than not carry some residual infectious NNV in their system that may proliferate rapidly once these fish are confronted with abiotic disease-causing agents such as abrupt temperature changes and abnormal water physicochemical parameters (Gomez *et al*., 2004; Mushiake *et al*., 1994, 1992).

In our previous bioassay experiments in tanks, we successfully demonstrated the efficacy of the formalin-inactivated Philippine strain of NNV in sea bass, groupers, and pompano juveniles against experimental challenges, i.e. via injection and immersion, with the homologous NNV (Pakingking *et al*., 2011, 2010, 2009). We also noted that a potent anamnestic response would arise when vaccinated groupers that survived NNV challenge were re-exposed to infectious NNV as indicated by substantial increases, i.e. up to six-folds or higher, in NNV-neutralizing antibody titers in NNV re-challenged fish (Pakingking *et al*., 2010). Although our tank studies have clearly demonstrated the efficacy of the formalin-inactivated Philippine strain of NNV vaccine against experimental NNV challenge, however, its field efficacy has not yet been thoroughly investigated. Thus, in this context, the field efficacy of the formalin-inactivated Philippine strain of NNV vaccine was tested in orange-spotted grouper, a highly susceptible fish species to VNN, reared in floating net cages in earthen pond in Dumangas Brackish Water Station (DBS) of the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC/AQD). Specifically, we examined the immunogenicity and protective immunity of the inactivated-NNV vaccine in the context of its ability to induce the production of NNV-neutralizing antibodies and conferment of protection in fish against artificial NNV challenge.

Materials and methods

Fish

A total of 210 healthy orange-spotted grouper juveniles (*E. coioides*) with mean body weight (MBW) of 8 g, obtained from a private hatchery in Roxas City, Capiz, Philippines, were stocked in 1000-L tank supplied with sand-filtered and flow-through seawater at 28 ˚C. Fish were allowed to acclimate for 2 weeks before the start of the experiment. They were fed SEAFDEC/AQD formulated diet (3 %

body weight) once a day throughout the experiment. Prior to the conduct of the vaccination experiment, brain samples of 10 randomly chosen fish were aseptically dissected and screened for NNV by nested-step RT-PCR (Nishizawa *et al*., 1994). All samples examined were negative for NNV.

Virus strain

The OSGBF1E strain (Genotype: red spotted grouper nervous necrosis virus; RGNNV) of NNV was used as vaccine immunogen in the preparation of the vaccine (Pakingking *et al*., 2009). NNV was propagated in E-11 cells (Iwamoto *et al*., 2000) using L-15 medium supplemented with 2 % fetal bovine serum (FBS).

Preparation of the vaccine

The formalin-inactivated vaccine was prepared following the published method of Pakingking *et al*. (2009, 2010, 2011). Briefly, the OSGBF1E strain at a titer of $10^{9.2}$ $TCID_{50}$ ml^{-1} was inactivated with 0.5 % formalin, followed by incubation at 4 ˚C for 10 days. The vaccine was ascertained to be completely inactivated, i.e. free of any residual infective virus, by showing no CPE in E-11 cells after three serial passages.

Vaccination

Vaccination of groupers was carried out by first anesthetizing fish in holding aquaria (500 L) with MS222. Two hundred grouper juveniles (MBW: 8.3±1.2 g) were randomly divided into two groups. The first group composed of 100 fish were intraperitoneally (IP) injected with 100 µl of the vaccine (pre-inactivation titer: $10^{9.2}$ $TCID_{50}$ ml^{-1}) and stocked in 2×3×1.0 m floating net cage in the pond. Correspondingly, the same number of individuals were also injected with an equal volume of L-15 medium (control group). At different time points post-vaccination, i.e. Days 30, 60, 90, 120, and 150, blood samples were collected from the caudal veins of 5 fish randomly collected from each of the cages. Quantification of the neutralizing antibody titers in the sera of fish were done following the method described in the succeeding section. Both vaccinated and unvaccinated fish were monitored periodically for any signs of VNN.

Neutralizing antibody assay

Neutralizing antibody titers in the sera of both vaccinated and unvaccinated fish were quantified using the method of Pakingking *et al*. (2011, 2010, 2009, 2009). At each sampling time, fish (n=5) were randomly collected from each of the vaccinated and unvaccinated group, anesthetized, followed by the collection of blood from the caudal vein of fish. After allowing the blood to clot at 4 ˚C overnight, the serum was obtained by centrifugation at 1500 × g for 15 min. The serum was then divided into several aliquots and stored at -20˚C until used. To quantify the NNV-neutralizing antibody titer in the sera of fish, a seroneutralization assay was conducted following the method of Pakingking *et al*. (2011, 2010, 2009). Briefly, fish sera were diluted with 39 volumes of Hanks' balanced salt solution supplemented with penicillin (100 IU ml^{-1}) and streptomycin (100 µg ml^{-1}) (HBSS-PS). They were then diluted twofold with HBSS-PS and mixed with an equal volume (50 µl) of the viral suspension (50 µl, 10^2 $TCID_{50}$). Immediately after incubating the mixture at 25 ˚C for 60 min, aliquots of each mixture were inoculated into four wells of the 96-well plate seeded with E-11 cells at approximately 80 % confluency. Cytopathic effect (CPE) was observed daily for ten days and the NNV-neutralizing antibody titer was calculated according to Reed and Muench (1938).

Virus challenge

At day 30 and 120 post-vaccination or L-15 injection, twenty and ten fish respectively

from each vaccinated and unvaccinated group were randomly collected and brought to the Infection Building, Tigbauan Main Station, SEAFDEC/AQD. These fish were intramuscularly challenged with NNV at an inoculum dose of $10^{6.5}$ $TCID_{50}$/ fish and periodically observed for 14 days post-NNV challenge. Brains and kidneys of dead and surviving fish were aseptically collected and subjected to virus titrations. Prior to dissection, blood samples were taken from the caudal vein of surviving fish for NNV-neutralizing antibody detection.

Virus titrations

NNV titers in the brains of dead and surviving fish were quantified following a protocol adapted from the previous study of (Pakingking *et al*., 2011, 2010, 2009).

NNV detection in fish by RT-PCR amplification

The brains of vaccinated and unvaccinated fish at the termination of the experiment were examined by nested-step RT-PCR (Nishizawa *et al*., 1994).

Statistical analysis

Statistical analysis was carried out using Fisher's exact probability test for fish mortalities and Mann Whitney's U-test for neutralizing antibody titers (Pakingking *et al*., 2010).

Results and discussion

The field efficacy of the inactivated Philippine strain of NNV vaccine in orange-spotted grouper juveniles, a highly susceptible fish species to VNN, via intraperitoneal (IP) injection was investigated in the current study. We employed the IP injection because this mode of vaccine administration has been proven efficient in upregulating the immune system of fish to produce potent NNV-neutralizing antibodies as documented in our previous reports (Pakingking *et al*., 2018, 2011, 2010, 2009). Accordingly, we were able to establish and compare the kinetics of NNV-neutralizing antibody productions in vaccinated groupers reared in floating net cages in earthen pond at different time points post-vaccination and with our previous data generated on tank trials, respectively (Pakingking *et al*., 2011, 2010, 2009).

The result of seroneutralization assay conducted prior to the IP injection of groupers with either L-15 (control) or inactivated NNV vaccine showed the absence of NNV-neutralizing antibodies (<1:80) in the sera of fish. However, at Day 30 post-vaccination, potent NNV-neutralizing antibodies (mean titer: 1:1792±701) were detected in the sera of fish (n=5) that peaked at Day 60 (1:6656±3435) and thereafter started to gradually decline but still detectable at Day 150 (1:704±351) post-vaccination (**Figure 2**). On the contrary, NNV-neutralizing antibodies were not detected (<1:80) in groupers examined at scheduled intervals post-L15 injection. By far, the mean NNV-neutralizing antibody titers in the sera of orange spotted groupers quantified at different time points post-vaccination in the current study strongly corroborate with the results of our previous tank trials in brown marbled groupers (Pakingking *et al*., 2010). These results clearly indicate that the inactivated Philippine strain of NNV is highly immunogenic to groupers as evidenced by high levels of NNV-neutralizing antibodies in the sera of vaccinated fish. Notably, no significant differences were noted in the mean body weights between the vaccinated and unvaccinated fish examined at different time points post-vaccination or L-15 injection (**Figure 1**), clearly suggesting that the vaccine has no inadvertent effect on the growth of fish.

Because natural occurrence of VNN in net-caged groupers in earthen pond in DBS was not encountered during the course of the pond experiment despite several cases of VNN in grouper juveniles encountered in the same area in the past, the field efficacy of the inactivated vaccine in grouper could not be clearly elucidated. Thus, to circumvent this problem, representative samples from both vaccinated and unvaccinated fish were randomly collected from the pond, brought to TMS of SEAFDEC/AQD and were subsequently challenged via intramuscular injection with the homologous virus at a dose of $10^{6.5}$ TCID50/ fish at Day 30 post-vaccination. As a result, 25 % and nil mortality were obtained in unvaccinated and vaccinated fish, respectively (**Figure 3**). The survival rate obtained in pond-reared vaccinated fish challenged with the homologous NNV was evidently in consonance with the data that we obtained in our previous tank trials in brown marbled grouper (Pakingking *et al*., 2010). However, the mortality rate (25 %) obtained in Day 30 post-L-15 injected fish in the current study is lower compared with the result, i.e. 70 %, of our previous NNV challenge in brown marbled grouper similarly conducted at Day 30 post-L-15 injection. The difference observed in our previous and current study could be attributed to various factors including the body weight, age, species, and rearing conditions of the fish, and infectivity of the NNV used in the challenge experiments among others. For instance, in our previous report, the mean body weight (MBW) of tank-reared brown marbled groupers intramuscularly challenged with NNV at Day 30 post-L15 injection that resulted in 70 % mortality was only 12±2 g whereas in the current study, the MBW of orange-spotted groupers challenged with NNV that resulted in 25 % mortality was significantly higher, i.e. 21±1 g. Moreover, this hypothesis pertinent to the weight-dependent susceptibility of groupers to NNV is further backed up by nil mortality obtained in groupers with a mean body weight of 176±19 g that were challenged with NNV at Day 120 post-L-15 injection (de la Peña *et al*., 2017). It is worth noting that approximately 50 % of the Day 120 L15-injected groupers challenged with NNV at $10^{6.5}$ $TCID_{50}$/fish commenced manifesting lethargy at Day 3 followed by abnormal swimming behavior at Day 4 post-NNV injection (**Table 1**). The abnormal swimming behavior however lasted for only about 7 days after NNV challenge. Surprisingly, these fish gradually resumed normal swimming behavior and apparent recovery at the termination of the experiment. High NNV titers (>10^9 $TCID_{50}$/g) were detected in the brains of dead unvaccinated fish (**Table 1**). On the contrary, NNV was not detected in the brains of surviving vaccinated fish challenged with NNV (**Table 1**).

In summary, single administration of the monovalent formalin-inactivated NNV vaccine can effectively upregulate the production of NNV-neutralizing antibodies and concomitant conferment of protection against VNN in groupers especially during the early phase of grow-out culture in earthen ponds when these fish species are highly susceptible to the disease. Additionally, our current data also indicate the potential use of this inactivated vaccine against NNV infection in other grouper species and other warm-water marine fish species such as sea bass and pompano, particularly during the early phase of grow-out culture in earthen ponds or floating net-cages in the open sea, since mortalities of these fish species have been found to be caused by NNV strains belonging to a single genotype, i.e. RGNNV type.

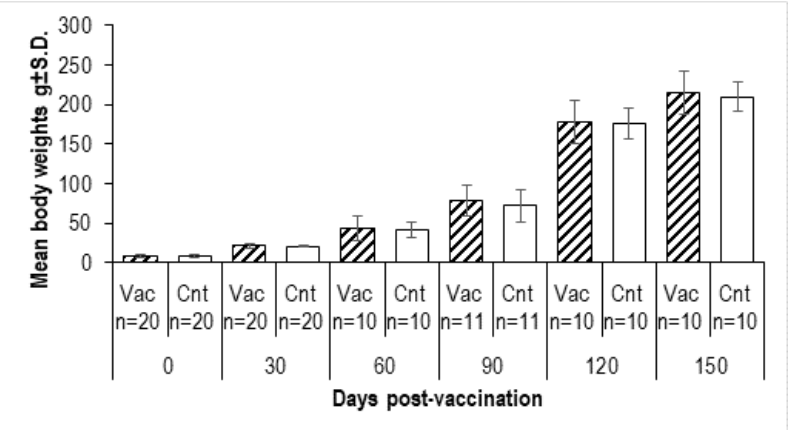

Figure1. Body weights (Mean ± SD) of vaccinated and unvaccinated orange-spotted groupers (*Epinephelus coioides*) examined at different time points post-vaccination

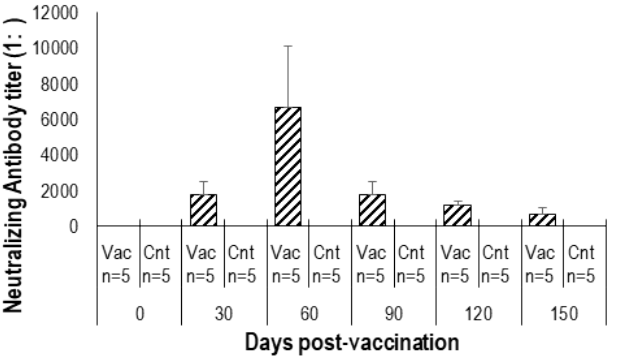

Figure 2. Neutralizing antibody titer (Mean ± SD) in the sera of vaccinated (Vac) and unvaccinated (Cnt) orange-spotted groupers (*Epinephelus coioides*) examined at different time points post-vaccination. The lowest detection limit is 1:80

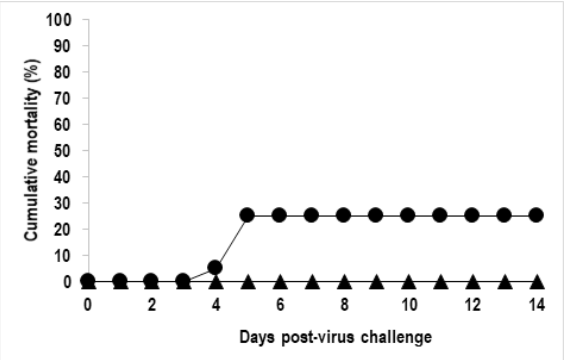

Figure 3. Cumulative mortalities of unvaccinated (●) and vaccinated (▲) orange-spotted grouper (*Epinephelus coioides*) juveniles intramuscularly injected with nervous necrosis virus at an inoculum dose of $10^{6.5}$ $TCID_{50}$ $fish^{-1}$.

Table 1. Cumulative mortality, serum antibody titers and nervous necrosis virus (NNV) titers in orange-spotted groupers intramuscularly challenged with NNV (106.5 TCID50 fish-1) at Days 30 and 120 post-vaccination or L-15 injection (control).

Days post vaccination	No. of fish that died of NNV infection/No. of fish challenged with NNV (%) [Mean body weight (g)± SD]		Antibody titer before the NNV challenge (No. of fish examined)		Antibody titer 1 month after the NNV challenge (No. of fish examined)		No. of fish that exhibited abnormal swimming behavior & dark skin coloration*/ No. of fish challenged with NNV (%)		No. of surviving fish that recovered from abnormal swimming behavior & dark skin coloration**/ No. of fish that survived NNV challenge (%)		NNV Titer $Log_{10}TCID_{50}\ g^{-1}$ in the brains of surviving fish examined at 1 month post NNV challenge (No. of positive / total no. of fish examined)	
	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
Day 30	0/20 (0) [21±3.4]	5a /20 (25%) [21±1]	1792±701 (5)	<80 (5)	8192±2804 (5)	576±143 (5)	0/20 (0)	13/20 (65)	NA (0)	8/15 (53)	—[b] (0/5)	4.42 ± 0.02^{b} (5/5)
Day 120	0/20 (0) [178±27]	0/20 (0) [176±19]	1152±286 (5)	<80 (5)	14336±5609 (5)	704±351 (5)	0/20 (0)	6/20 (30)	NA (0)	6/20 (30)	—[b] (0/5)	4.35 ± 0.42^{b} (5/5)

* abnormal swimming behavior & dark coloration commenced from Day 3 to Day 4 post-NNV challenge; ** abnormal swimming behavior & dark coloration started to disappear from Day 8 post-NNV challenge; *** [a] Virus titer in the brains of dead fish (n=5) $10^{11.32\pm0.1}\ TCID_{50}g^{-1}$; [b] $<10^{2}\ Log_{10}TCID_{50}\ g^{-1}$; NA, not applicable

Acknowledgment

This study was funded by Government of Japan Trust Fund VI through the Regional Fish Disease Project (study code: FH01-F2015-T) and in part by SEAFDEC/ AQD. We express our heartfelt gratitude to Dr. Takuro Shibuno and Dr. Chihaya Nakayasu, former GOJ-TF managers, Dr. Koh Ichiro Mori, current GOJ-TF manager, and the Marine Fish Hatchery staff especially Mr. A. Gamuza.

References

Bandín, I., Souto, S. 2020. Betanodavirus and VER Disease: A 30-year Research Review. Pathogens 9. https://doi.org/10.3390/pathogens9020106

Comps, M., Pépin, J.F., Bonami, J.R. 1994. Purification and characterization of two fish encephalitis viruses (FEV) infecting *Lates calcarifer* and *Dicentrarchus labrax*. Aquaculture 123, 1–10. https://doi.org/10.1016/0044-8486(94)90114-7

de la Peña, L.D., Suarnaba, V.S., Jinky, A., Villacastin, B., Cabillon, N.A.R., Catedral, D.D., Faisan Jr, J.P. 2017. Susceptibility of Different Weight Ranges of *Epinephelus coioides* to Piscine Nodavirus. Bull. Eur. Ass. Fish Pathol 37, 16–22.

Gomez, D.K., Sato, J., Mushiake, K., Isshiki, T., Okinaka, Y., Nakai, T. 2004. PCR-based detection of betanodaviruses from cultured and wild marine fish with no clinical signs. J. Fish Dis. 27, 603–608. https://doi.org/10.1111/j.1365-2761.2004.00577.x

Iwamoto, T., Mise, K., Takeda, A., Okinaka, Y., Mori, K.-I., Arimoto, M., Okuno, T., Nakai, T. 2005. Characterization of Striped jack nervous necrosis virus subgenomic RNA3 and biological activities of its encoded protein B2. J. Gen. Virol. 86, 2807–2816. https://doi.org/10.1099/vir.0.80902-0

Iwamoto, T., Nakai, T., Mori, K., Arimoto, M., Furusawa, I. 2000. Cloning of the fish cell line SSN-1 for piscine nodaviruses. Dis. Aquat. Org. 43, 81–89. https://doi.org/10.3354/dao043081

Maeno, Y. (Japan I.R.C. for A.S., de la Pena, L.D., Cruz-Lacierda, E.R. 2002. Nodavirus infection in hatchery-reared orange-spotted grouper *Epinephelus coioides*: First record of viral nervous necrosis in the Philippines. Fish Pathology (Japan).

Maeno, Y., Peña, L.D.D.L., Cruz-Lacierda, E.R. 2004. Mass Mortalities Associated with Viral Nervous Necrosis in Hatchery-Reared Sea Bass *Lates calcarifer* in the Philippines. Japan Agricultural Research Quarterly: JARQ 38, 69–73. https://doi.org/10.6090/jarq.38.69

Mori, K. (Japan S.F.A., Mushiake, K., Arimoto, M. 1998. Control measures for viral nervous necrosis in striped jack (Pseudocaranx dentex). Fish Pathology (Japan).

Mori, K., Nakai, T., Muroga, K., Arimoto, M., Mushiake, K., Furusawa, I. 1992. Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. Virology 187, 368–371. https://doi.org/10.1016/0042-6822(92)90329-n

Mushiake, K., Arimoto, M., Furusawa, T., Furusawa, I., Nakai, T., Muroga, K. 1992. Detection of antibodies against striped jack nervous necrosis virus (SJNNV) from brood stocks of striped jack. Nippon Suisan Gakkaishi. URL http://ir.lib.hiroshima-u.ac.jp/00025668 (accessed 6.11.20).

Mushiake, K., Nishizawa, T., Nakai, T., Furusawa, I., Muroga, K. 1994. Control of VNN in Striped Jack : Selection of Spawners Based on the Detection of SJNNV Gene by Polymerase Chain Reaction (PCR). Fish Pathology 29, 177–182. https://doi.org/10.3147/jsfp.29.177

Nakai, T., Sugaya, T., Nishioka, T., Mushiake, K., Yamashita, H. 2009. Current Knowledge on Viral Nervous Necrosis (VNN) and its Causative Betanodaviruses.

Nishizawa, T., Furuhashi, M., Nagai, T., Nakai, T., Muroga, K. 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Appl. Environ. Microbiol. 63, 1633–1636.

Nishizawa, T., Mori, K., Nakai, T., Furusawa, I., Muroga, K. 1994. Polymerase chain reaction (PCR) amplification of RNA of striped jack nervous necrosis (SJNNV). Diseases of Aquatic Organisms. https://doi.org/10.3354/dao018103

Pakingking, R., Bautista, N.B., de Jesus-Ayson, E.G., Reyes, O. 2010. Protective immunity against viral nervous necrosis (VNN) in brown-marbled grouper (*Epinephelus fuscoguttatus*) following vaccination with inactivated betanodavirus. Fish Shellfish Immunol. 28, 525–533. https://doi.org/10.1016/j.fsi.2009.12.004

Pakingking, R., de Jesus-Ayson, E.G., Reyes, O., Brian Bautista, N. 2018. Immunization regimen in Asian sea bass (*Lates calcarifer*) broodfish: A practical strategy to control vertical transmission of nervous necrosis virus during seed production. Vaccine 36, 5002–5009. https://doi.org/10.1016/j.vaccine.2018.07.015

Pakingking, R., Mori, K.-I., Bautista, N.B., de Jesus-Ayson, E.G., Reyes, O. 2011. Susceptibility of hatchery-reared snubnose pompano *Trachinotus blochii* to natural betanodavirus infection and their immune responses to the inactivated causative virus. Aquaculture 311, 80–86. https://doi.org/10.1016/j.aquaculture.2010.11.035

Pakingking, R., Seron, R., dela Peña, L., Mori, K., Yamashita, H., Nakai, T. 2009. Immune responses of Asian sea bass, *Lates calcarifer* Bloch, against an inactivated betanodavirus vaccine. J. Fish Dis. 32, 457–463. https://doi.org/10.1111/j.1365-2761.2009.01040.x

Reed, L.J., Muench, H. 1938. A Simple Method of Estimating Fifty Per Cent Endpoints. Am J Hygiene 27, 493–497. https://doi.org/10.1093/oxfordjournals.aje.a118408

Sommerset, I., Nerland, A.H. 2004. Complete sequence of RNA1 and subgenomic RNA3 of Atlantic halibut nodavirus (AHNV). Dis. Aquat. Org. 58, 117–125. https://doi.org/10.3354/dao058117

Thiery, R., Johnson, K., Nakai, T., Schneemann, A., Bonami, J.R., Lightner, D. 2012. Family - Nodaviridae, in: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy. Elsevier, San Diego, pp. 1061–1067. https://doi.org/10.1016/B978-0-12-384684-6.00092-6

Application of Carriers and RNAi to Enhance the Antiviral Immune Response of Shrimp to WSSV

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Abstract

In aquaculture, vaccination is one of the approaches for disease prevention and control. The aim of the present study was to determine the efficacy of a VP28 double stranded RNA (VP28 dsRNA) and recombinant VP28 protein (rVP28) administered together as an antiviral treatment against WSSV. Double-stranded RNA was produced in RNAse-deficient *Escherichia coli* HT115 following published methods. To determine the appropriate dose, different concentrations of dsRNA ranging between 0.2 µg and 20 µg, were either injected intramuscularly or delivered orally to the shrimp via the feed ration. Thereafter, the shrimp were challenged with WSSV either by injection ($LD_{50}=10^{-7}$ dilution of the gill tissue filtrate) or bath immersion ($LD_{50}=10^{-4}$ dilution of the filtrate) in glass aquaria and transferred to fiberglass tanks for daily monitoring and recording of mortalities. Results showed significant differences in survival between PBS and the 0.2, 10, and 20 µg dsRNA/shrimp doses. Time to 100 % mortality significantly differed among the treatments with the control reaching mortality earlier (day 4) while shrimp injected with 0.2 and 10 µg dsRNA succumbed to WSSV much later on days 9–12. Different frequencies of dsRNA administration were also tested. The best result obtained was a dose of 20 µg/shrimp administered 4 times over 28 days (2 times before and 2 times during challenge for a total 80 µg/shrimp). Finally, VP28 dsRNA was combined with rVP28 at ratios of 1:1, 1:2, 1:3, 3:1, and 2:1, entrapped in chitosan microparticles and delivered per os via the feed according to the dose and frequency as previously determined. Following bath exposure challenge with WSSV, the best survival obtained in trials 1 and 2 was 40 % and 43 % at 1:3 VP28 dsRNA to rVP28 ratio.

Introduction

In shrimp aquaculture, a safe, effective, and inexpensive antiviral treatment is required to limit the impact of WSSV and other shrimp viruses. Successful vaccination in shrimp using whole formalin-killed virus or recombinant protein or plasmid DNA has been reported (Witteveldt et al., 2004; Rout et al., 2007). Vaccine vectors or carriers are examined based on their safety profile, economics, and practical utility. Vaccine carriers that protect antigens from degradation, ensure release of adequate antigen dose, contain immunomodulatory substances, and suitable for oral delivery in commercial grow-out systems such as ponds are deemed appropriate. Alginate and chitosan microparticles were previously tested as microparticle drug delivery systems in fish and freshwater prawn (Rodrigues

et al., 2006; Anas et al., 2008) and as recombinant vaccine carrier for giant tiger shrimp (*manuscript in preparation*). Owing to their properties, microparticles can deliver drugs or antigens comparable to or better than non-encapsulated inactivated whole virus when added to feed rations (Amar and Faisan, 2011; Amar *et al*., 2021). RNAi is an emerging technology that is based on gene silencing (Sagi *et al*., 2013). The silenced gene, by degrading its mRNA, is unable to produce the protein that performs an essential function. It has been applied in aquaculture in sex manipulation and control of reproduction (Ventura *et al*., 2009; Treerattrakool *et al*., 2011; 2013) and lately in disease control in crustaceans (Xu *et al*., 2007; Escobedo-Bonilla, 2011; Le Fauce and Owens, 2012). The antiviral effect of RNAi is based on silencing a viral or host gene that is primarily involved in viral pathogenesis. The main constraint of RNAi as an antiviral agent is production cost and a practical method of delivery. Thus, methods to reduce production cost of dsRNA as well as application of the microparticle method of delivery were examined. Moreover, a scheme where the two treatments are combined to enhance efficacy was explored. The main objective of the study was to apply emerging technologies in the management of WSSV infections in shrimp. Specifically, the study aimed to evaluate the efficacy of dsRNA treatment in protecting shrimp against WSSV, develop a prophylactic scheme combining the two treatments (rVP28+rVP28 dsRNA), and develop an inexpensive vaccine/drug delivery protocol for WSSV prevention in tanks and in ponds-based culture systems.

Materials and methods

Isolation of primary lymphoid organ (LO) cells and observation of CPE

Primary shrimp cells were isolated from lymphoid organ following the method of Assavalapsakul *et al*. (2003) with modifications. Briefly, the lymphoid organ was excised and pushed gently against a 100 µ mesh steel screen with a rubber-tipped syringe plunger to force the cells onto a sterile petri dish with Leibovit's (L15) medium. All materials used were sterilized by autoclaving. The isolated cells were washed several times in L15 by repeated pipetting and centrifugation, and the final cell pellet was suspended in L15 and counted under a microscope with a haematocytometer. Shrimp cells were plated in 96-well plate at an estimated density of 10^6 cells ml^{-1}. Then, 10^0-10^{-3} dilutions of the virus supernatant from homogenates of infected gill tissue were added and the cells were observed for the development of CPE for 7 days. The negative control wells contained cells without the virus. The number of cytopathic foci were assessed microscopically under 400x magnification.

Preparation of double-stranded RNA

Double-stranded RNA was prepared using a low-cost *in vivo* bacterially expressed dsRNA production method described by Ongvarrasopone et al. (2007). In this method, a strain of *E. coli* lacking RNAse III (HT115) was transformed with a plasmid containing the T7 RNA polymerase promoter and a DNA sequence (VP28

gene) homologous to a target viral protein (GenBank accession no. AF380842). The bacteria was then cultured and induced by IPTG to produce dsRNA that was then extracted from the bacterial cell by a combination of boiling in 0.1 % SDS, and protease and RNAse treatment to remove protein, single stranded RNA and total RNA of the host cell (**Figure 1**). The dsRNA was quantified using a nano spectrophotometer.

Evaluation of the efficacy of RNAi in protecting shrimp against WSSV infection

The efficacy of dsRNA treatments (both by intramuscular injection and by oral administration) was tested *in vivo* in tanks trials. dsRNA (100 µl) was first injected to 10 g shrimp at 0.2 and 10 µg/shrimp. Twenty-four hours after dsRNA injection, shrimp were injected with the virus (100 µl of 10^{-7} dilution of the infected gill tissue supernatant (LD_{50} as determined by the method of Reed and Muench (1938) from an earlier study) and returned to the tanks for observation and recording of mortality for 10 days. Semi-quantitative determination of the viral load by PCR in the hemolymph of the treated and control shrimp was also performed. The mean mortality values of dsRNA-treated and control shrimps were compared. Based on the results of the first trial, trial 2 was conducted using 0.2, 10, and 20 µg/shrimp (15 g) but instead of injection, dsRNA was administered orally through the feed for 14 days, and the shrimp challenged with WSSV by immersion at a dilution of 10^{-4} of the gill tissue supernatant (immersion LD_{50} as determined earlier). Finally, trial 3 was conducted using the best treatment obtained in trial 2 (20 µg/shrimp x 2 times before challenge and 2 times after challenge for a total of 80 µg/shrimp for 28 days). Different frequencies (8x, daily, 4x at 20 and 30 µg/shrimp over 28 days) were tested to determine whether a dose given once or divided into several smaller doses would result in better survival upon immersion challenge. A control group without dsRNA treatment and *E. coli* dsRNA were added to account for non-specific dsRNA treatment effects.

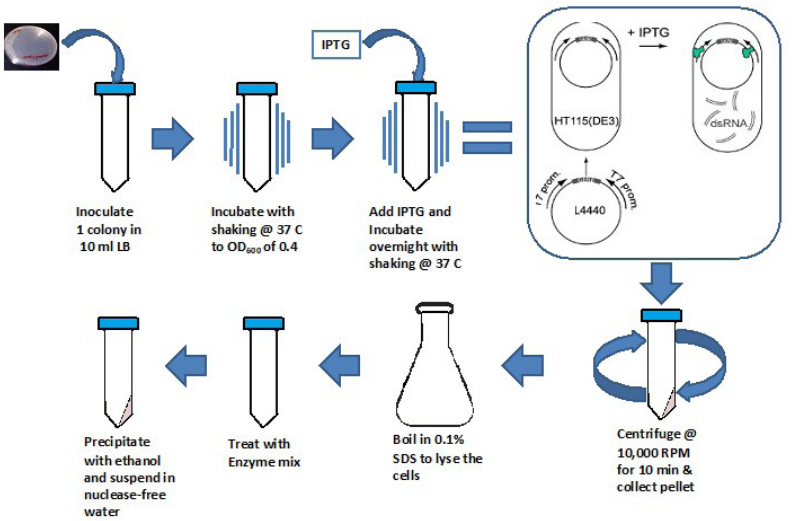

Figure 1. Preparation of VP28 double-stranded RNA. Cloning and transformation (a); culture of bacteria and extraction of bacterially-expressed dsRNA

Preparation of alginate and chitosan microparticles

Microparticles were prepared by ionotropic gelification following the method of Aral and Akbuga (2003) which was adapted and modified for the project. Briefly, chitosan solution was prepared by dissolving 0.35 g chitosan powder in 100 ml 1 % v/v Tween 80 and 2 % v/v Acetic acid, stirred for faster solubilization, and the dsRNA and/or solubilized inclusion bodies containing the VP28 protein were added. Ten milliliters 20 % sodium sulfate was added drop by drop and stirred continuously for 1h at the highest possible speed. The mixture was then transferred to a 50 ml blue cap tube and centrifuged at 1100 g for 30 min at room temperature (8,237 rpm in CR 21G centrifuge). The supernatant was decanted and the weight of the microparticles produced was determined. Alginate microparticles were prepared according to Rodriguez et al. (2006) and Tian et al. (2008). Alginate was dissolved in distilled water (3 % w/v) with dsRNA and/or solubilized rVP28 inclusion bodies. This is the aqueous phase. The oil phase was 2 % w/v Tween 80 in vegetable oil, whereas the gelification solution was 6.8 g CaCl2.2H2O+ 30 ml distilled water+ 30 ml ethanol+ 2 ml glacial acetic acid. The alginic suspension with dsRNA and protein was poured to 20 ml oil mixture to form the AS+P+O mix and stirred at maximum speed for 10 min in a magnetic stirrer. The AS+P+O mix was added to the gelification solution and stirred at maximum speed for 30 min. Layers were allowed to separate for at least 2 h. The top oil layer was removed by pipetting and the aqueous layer was centrifuged at 500 g for 25 min at 25°C to obtain the alginate microparticles. Microparticles were stored at 4°C until use. Encapsulation efficiency, loading percentage and yield of microsphere were evaluated both for alginate and chitosan microparticles using albumin as standard protein.

Tank evaluation of encapsulated dsRNA with rVP28 vaccine

A combined VP28 dsRNA and rVP28 protein vaccine encapsulated in alginate and chitosan microparticles and delivered orally through the diet was next tested to determine the proportion of rVP28 vaccine to VP28 dsRNA that could enhance overall efficacy of the treatment. This was done by incorporating the encapsulated dsRNA+ vaccine into the feeds, followed by feeding for 2 weeks at a dose determined earlier, tank challenge trial, and feeding for another 2 weeks until completion of the challenge test. Recombinant VP28 vaccine was prepared as described in previous reports. Encapsulation of VP28 dsRNA+rVP28 vaccine was done at different dsRNA to protein ratios using both alginate and chitosan microparticles. The WSSV challenge was conducted by bath-immersion in 1 LD_{50} or 10^{-4} dilution of the infected gill tissue supernatant.

Statistical analysis

Unless otherwise stated, data were expressed as means of 3 replicates ± standard error of the mean. The data were analyzed with Analysis of Variance (ANOVA) with post-hoc multiple comparison of means by Tukey's Highly Significant Difference Test (Tukey's-HSD). Percentage data were checked for normality and were arcsine-transformed before analysis. Differences were considered statistically significant when $p<0.05$.

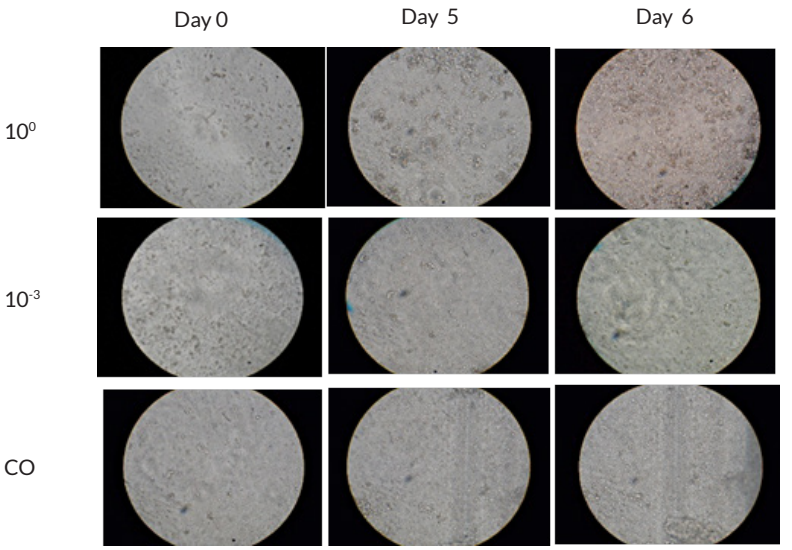

Figure 2. Primary cells of the *P. monodon* lymphoid organ of showing CPE upon in vitro infection with 10^0 and 10^{-3} dilution WSSV

Results

Isolation of primary lymphoid organ (LO) cells and observation of CPE

After 5 days of incubation, the cells showed signs of CPE (detachment from the well bottom and aggregation) due to WSSV infection. CPE was clearly found at 10^0 to 10^{-3} dilutions of the WSSV tissue filtrate whereas the higher dilutions had inconclusive results (**Figure 2**). The cell cultures could not be successfully observed for longer periods because of contamination. As the results indicated the need for further optimization of the primary cell culture, it was decided that *in vivo* evaluation would be employed from then on.

Preparation of double-stranded RNA

The agarose gel electrophoresis of the VP28 DNA that was PCR amplified from WSSV-infected shrimp gill tissues, the EcoRV-digested pL4440 plasmid, and the gel band of the colony PCR of the transformed bacteria containing recombinant plasmid (HT115/pL4440VP28) are shown in **Figure 2**. **Table 1** shows the quantity of dsRNA produced by representative colonies using the commercial kit and the SDS method.

Evaluation of the efficacy of RNAi in protecting shrimp against WSSV infection

Initial results showed significant differences in survival between PBS and 0.2 and 10 µg/shrimp dsRNA dose on day 3-7 post-challenge. Time to reach 100 % mortality also significantly differed among treatments with the control reaching 100 % mortality on day 4 while shrimp that received 0.2 and 10 µg/shrimp eventually died on days 9-12 (**Figure 3**). In trial 2, the best treatment was 20 µg/shrimp delivered 4x (2 x before challenge and 2x during challenge) which had 70 % cumulative mortality and significantly different from the control. Although not all shrimp died, those given 0.2 and 10 µg/shrimp delivered

Table 1. Quantity of dsRNA (µg/ml) produced by representative colonies C1-C7 using a commercial RNA extraction kit and the long method of SDS and enzyme treatment

Colony	SV RNA Kit	0.1% SDS and enzyme treatment for medium-scale prep
C1	0.772	164.5
C2	0.831	160.6
C3	0.506	158.8
C4	0.589	149.5
C5	0.341	131.0
C6	0.371	141.8
C7	0.498	148.0

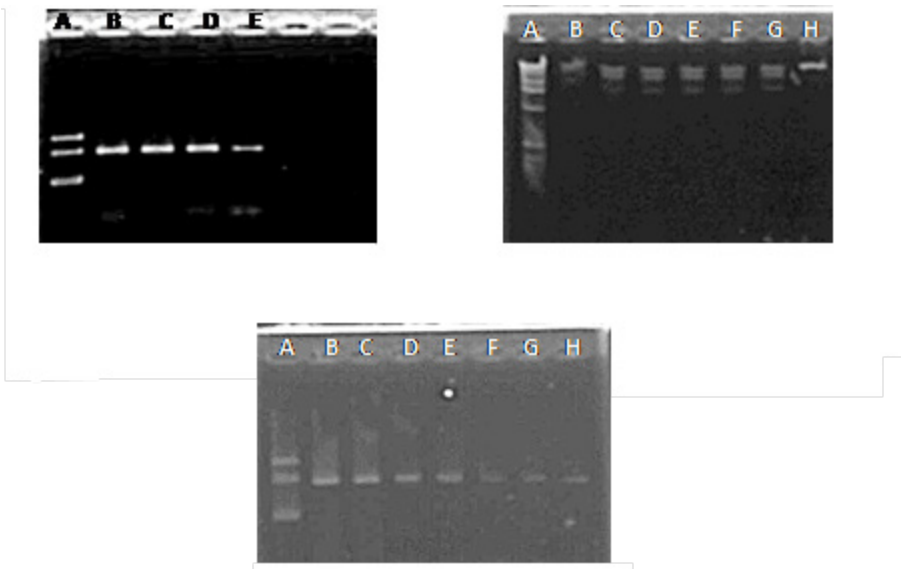

Figure 3. Agarose gel electrophoresis of VP28 DNA fragment after PCR of WSSV-infected gills of shrimp (A); EcoRV-digested pL4440 plasmid vector showing two bands (B-G) for the digested and one band only (H) for the undigested plasmid (B); and colony PCR of the transformed bacteria (HT115) harboring the recombinant plasmid pL4440VP28 (C)

4x (2 x before challenge and 2x during challenge) had survival that did not differ from the control (**Figure 4**). In trial 3, the frequencies from daily to 8 times over 28 days did not differ among treatments but these groups had cumulative mortalities ranging from 63-68% which were lower than the untreated control and unrelated dsRNA (100%) (**Figure 5**)

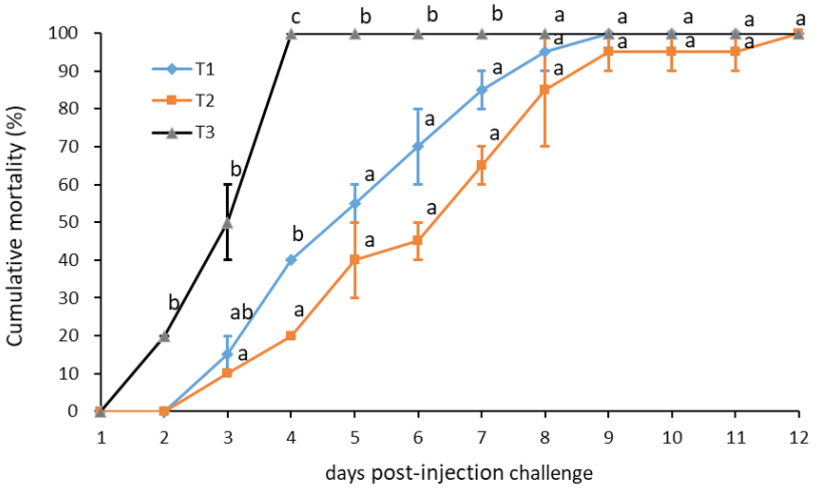

Figure 4. Cumulative mortality of the VP28 dsRNA-injected *Penaeus monodon* 12 days post-injection challenge with 1 LD_{50} of WSSV. T1, 0.2 µg/shrimp; T2, 10 µg/shrimp; T3, PBS. Line graphs represent means SEM (n=2 replicate tanks). Means at each time point with the same letter superscripts are not significantly different (p>0.05)

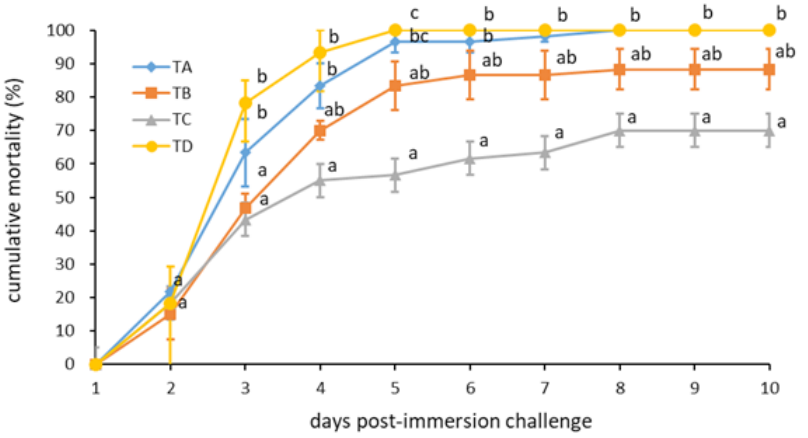

Figure 5. Cumulative mortality in *P. vannamei* fed with VP28 dsRNA and challenged with WSSV (TA, 0.2 µg/shrimp; TB, 10 µg/shrimp; TC, 20 µg/shrimp; TD, control). Line graphs represent means SEM (n=3 replicate tanks). Means with the same letter superscripts are not significantly different (p>0.05)

Preparation of microparticles and evaluation of their encapsulation efficiency, loading percentage, and yield

The chitosan and alginate microparticles as imaged by scanning electron microscope are shown in **Figure 6**. During the first 3 trials using albumin as the model protein, chitosan had 0.13 % loading percentage, 73.07 % encapsulation efficiency and 99.59 % yield as compared to alginate which had 0.21 %, 87.61 % and 98.72 %, respectively. Using both antivirals, alginate microparticles had higher encapsulation efficiency of 82.95 % as compared to 56.09 % for chitosan. Loading percentage for both antivirals was at 0.03 % and

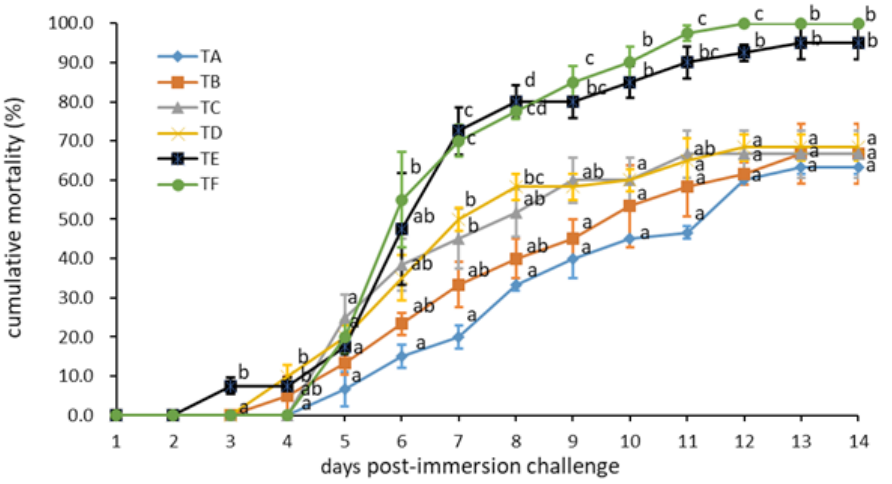

Figure 6. Cumulative mortality of *P. vannamei* fed dsRNA for 28 d at a single dose and different frequencies (TA, 2.86 µg/shrimp daily; TB, 10 µg/shrimp/ 8d; TC, 20 µg/shrimp/4d; TD, 30 µg/shrimp/4d; TE, E. coli non-specific dsRNA; TF, PBS. Line graphs represent means SEM (n=3 replicate tanks). Means at each time point with the same letter superscripts are not significantly different (p>0.05)

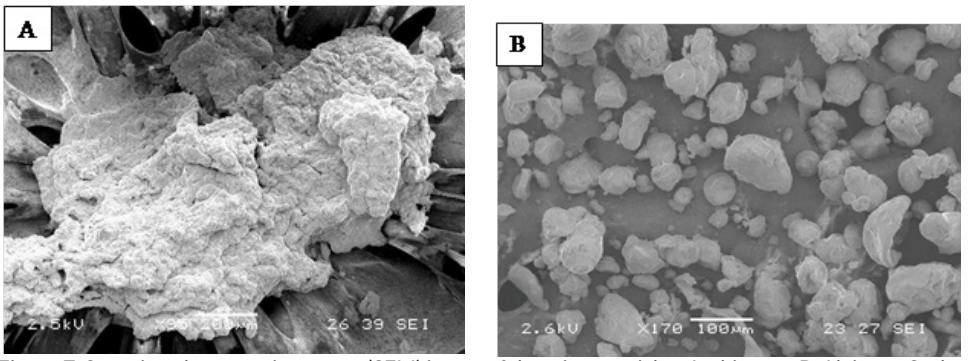

Figure 7. Scanning electron microscope (SEM) image of the microparticles. A, chitosan; B, Alginate. Scale bar: 100 µm

Table 2. Evaluation of the loading percentage, encapsulation efficiency, and yield of microspheres of dsRNA and rVP28 using chitosan and alginate. Albumin was used as the protein standard

	Chitosan	
Variables Calculated	**Albumin (%)**	**dsRNA and BL21 (%)**
Loading Percentage	0.13	0.03
Encapsulation Efficiency	73.07	56.09
Yield of Microspheres	99.59	99.97

	Alginate	
Variables Calculated	**Albumin (%)**	**dsRNA and BL21 (%)**
Loading Percentage	0.21	0.03
Encapsulation Effiency	87.61	82.95
Yield of Microspheres	98.72	99.98

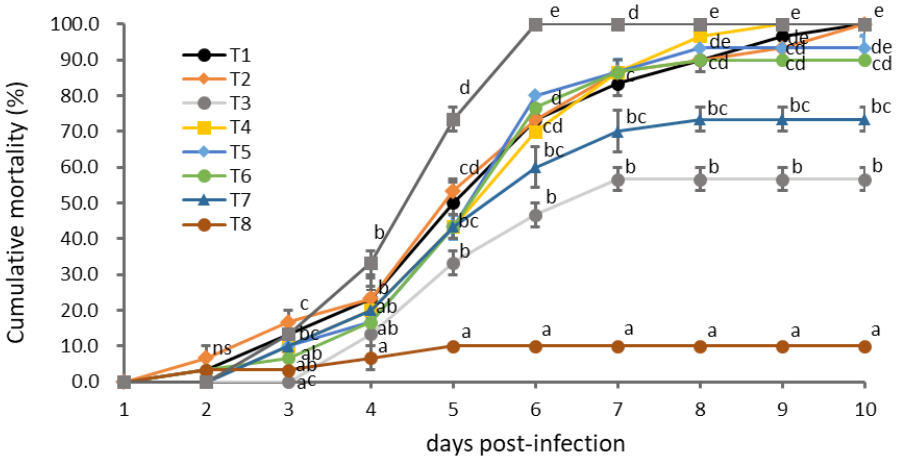

Figure 8. Cumulative mortality of *P. vannamei* fed for 28d at different VP28 dsRNA to rVP28 ratios: 1:1 (T1); 1:2 (T2); 1:3 (T3); 2:1 (T4); and 3:1 (T5); dsRNA only (T6); rVP28 only (T7); PBS Negative control (T8); virus positive control (T9). Trial 1. Line graphs represent means SEM (n=3 replicate tanks). Means at each time point with the same letter superscripts are not significantly different (p>0.05)

yield of microspheres was at 99.97 % for chitosan and 99.98 % for alginate (**Table 2**).

Survival of shrimp fed the combined VP28 dsRNA and rVP28 at different ratios

Survival of shrimp fed VP28 dsRNA plus rVP28 at different ratios ranging from 1:1 to 1:3 and 3:1 to 2:1 are shown in **Figure 7 (trial 1)** and **Figure 8** (**trial 2**). In both trials, shrimp fed dsRNA and protein at a ratio of 1:3 exhibited the highest survival after being challenged with WSSV at 1 immersion LD_{50} (10^{-4} dilution of the viral supernatant). However, in trial 1, this ratio had mortality that was significantly lower than dsRNA alone, the control, and the rest of the ratios but not lower than rVP28 alone (**Figure 7**). Similarly, in trial 2, the 1:3 ratio was significantly lower than the control but not lower than the rest of the treatments (**Figure 8**).

Discussion

In this study we found that dsRNA at a dose of 20 µg/shrimp administered 4x over 28 days gave the highest survival in shrimp challenged with 10^{-7} LD_{50} WSSV. For lower doses by injection of dsRNA, the results indicated that shrimp were protected from WSSV infection until day 7 post challenge. However, the shrimp eventually suffered 100 % mortality, although at different days post challenge. There are a few explanations for this result. The dsRNA dose might be too low

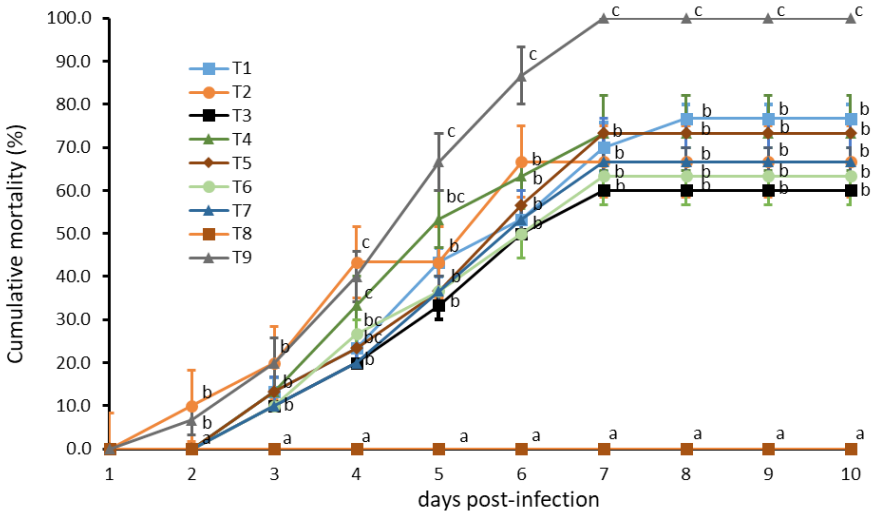

Figure 9. Cumulative mortality of *P. vannamei* fed for 28d at different VP28 dsRNA to rVP28 ratios: 1:1 (T1); 1:2 (T2); 1:3 (T3); 2:1 (T4); and 3:1 (T5); dsRNA only (T6); rVP28 only (T7); PBS Negative control (T8); virus positive control (T9). Trial 2. Line graphs represent means SEM (n=3 replicate tanks). Means at each time point with the same letter superscripts are not significantly different (p>0.05)

or that the injected dsRNA might not be able to persist in the tissue long enough to sustain its knockdown effect and exert protection. Against gill-associated virus, (GAV) injection but not oral administration protected shrimp from infection (Sellars et al., 2011), but oral administration was able to protect shrimp from WSSV (Sarathi et al., 2008). Subsequent experiments were then conducted with higher doses of dsRNA and frequency of administration. To examine if the persistence of dsRNA in the tissue might be affected by dose and frequency, dsRNA was administered orally by feeding it to the shrimp several times over the duration of the experiment. Also, the viral challenge method adopted was by bath-immersion as this method would likely give a more natural progression of mortality. Based on the results of the 3 trials, the best dose appeared to be 20 µg/shrimp administered 4x over 28 days. Increasing the frequency to 8 times or daily over 28 days did not further improve the results. The effect of dsRNA was specific to VP28 as non-specific *E. coli* dsRNA did not protect shrimp at all and had cumulative mortality similar to the untreated control. When VP28 dsRNA and rVP28 were combined, the best ratio obtained was 1part dsRNA to 3 parts rVP28 which exhibited the highest survival in the tank challenge trial.

The microparticle assay revealed that the alginate microparticles had a higher encapsulation efficiency (82.95 %) compared to chitosan microparticles (56.09 %) using both antivirals during the preliminary trials. Additionally, yield of microspheres and encapsulation efficiency for alginate was affected by the weight of the microparticle produced. This was due to the inability of alginate microparticles to separate from its liquid component and the weight of the microparticle produced was affected by the presence of entrapped liquid. Therefore, the higher values for encapsulation efficiency in alginate preparation was due to the increased wet weight of the microparticle produced. This result suggests that chitosan is a better microparticle to use when encapsulating both antivirals for incorporation into the shrimp feed.

A ratio of 1 part VP28 dsRNA to 3 parts rVP28 proved to be the best combination in terms of protecting the shrimp against WSSV infection. However, the combined treatment did not consistently improve the survival over dsRNA alone (significant difference was found only in trial 1) and did not improve survival over rVP28 alone (both trial 1 and 2). This could mean that addition of rVP28 improves the effect of VP28 dsRNA, but addition of dsRNA does not enhance the effect of rVP28. There was no direct comparison between the non-encapsulated and encapsulated VP28 dsRNA, rVP28 or their combination but our previous study estimated a 24–30 % increase in survival with the use of microparticle carriers and rVP28 (*manuscript in preparation*). Apart from potentially increasing survival, use of microparticle carriers could facilitate oral delivery of antiviral molecules such as dsRNA and protein without compromising their efficacy.

Conclusion

VP28 dsRNA was effective in reducing mortality due to WSSV infection in tank experiments. Based on the results of 3 trials, the best treatment was a dose of 20 µg/shrimp administered 4 times over 28 days before and during challenge for a total dose of 80 µg/shrimp. A higher dose and more frequent administration did not further increase survival. The resistance against WSSV challenge was specific to VP28 dsRNA as heterologous dsRNA gave no significant protection. The best ratio of VP28 dsRNA to rVP28 was found to be 1:3 which elicited 40–43 % protection in WSSV challenge tests. However, while addition of rVP28 significantly improved survival compared to VP28 dsRNA alone, addition of VP28 dsRNA did not significantly improve survival compared to rVP28 alone. A field efficacy evaluation of the microparticle-encapsulated VP28dsRNA and/or rVP28 by oral delivery (via feeding) in brackish water ponds is recommended.

Acknowledgment

We are indebted to the Government of Japan-Trust Fund for the grant funds (study code: FH-03-C2015T) and SEAFDEC/AQD management for the financial and moral support. We are also thankful to the Fish Health Section for assistance in various analyses.

References

Amar EC, Faisan JP, Jr. 2011. Efficacy of an inactivated vaccine and nutritional additives against white spot syndrome virus (WSSV) in shrimp (*Penaeus monodon*). *Israeli J Aquacult.-Bamidgeh*. 63:9 p.

Amar EC, Faisan, JP, Jr., Gapasin, RSJ. 2021. Field efficacy evaluation of a formalin-inactivated white spot syndrome (WSSV) vaccine for the preventive management of WSSV infection in shrimp grow-out ponds. *Aquaculture* 531: 735907.

Anas A, Philip R, Singh ISB. 2008. Chitosan as a wall material for a microencapsulated delivery system for *Macrobrachium rosenbergii* (de Man) larvae. *Aqua. Res.* 39: 885–890.

Aral C, Akbuga J. 2003. Preparation and in vitro transcription of chitosan microspheres containing plasmid DNA: poly L-lysine complexes. *J Pharm Pharmaceut Sci* 6:321-326.

Assavalapsakul W, Smith DR and Panyim S. 2003. Propagation of infectious yellow head virus particles prior to cytopathic effect in primary lymphoid cell cultures of *Penaeus monodon*. *Dis Aquat Organ* 55:253-258.

Escobedo-Bonilla CM. 2011. Application of RNA interference (RNAi) against viral infections in shrimp: A review. *J Antivir Antiretrovir* S9.doi:10.4172/jaa.S9-001.

La Fauce K, Owens L. 2012. RNA interference with special reference to combating viruses of crustacean. *Indian J. Virol.* 23:226- 243.

Ongvarrasopone C, Roshorm Y, Panyim S. 2008. A simple and cost-effective method to generate dsRNA for RNAi studies in invertebrates. *Science Asia* 33: 35-39.

Reed LJ, Muench H. 1938. A simple method of determining fifty percent endpoints. The Amer J Hygiene 27: 490-497.

Rodrigues AP, Hirsch D, Figueiredo HCP, Logato PVR, Moraes AM. 2006. Production and characterization of alginate microparticles incorporating *Aeromonas hydrophila* for fish oral vaccination. *Process Biochem* 41: 638-643.

Rout N, Kumar S, Jaganmohan S, Murugan V. 2007. DNA vaccines encoding viral envelop proteins confer protective immunity against WSSV in shrimp, *Penaeus monodon*. *Vaccine* 25: 2778-2786.

Sagi A, Rivka M, and Ventura T. 2013. Gene Silencing in crustaceans: from basic research to biotechnologies. *Genes* 4:620-645.

Sarathi M, Simon MC, Venkatesan C, Hameed ASS. 2008. Oral administration of bacterially expressed VP28dsRNA to protect *Penaeus monodon* from white spot syndrome virus. *Mar. Biotechnol.* 10: 242–249.

Sellars MJ, Rao M, Arnold SJ, Wade NM, Cowley JA. 2011. *Penaeus monodon* is protected against gill-associated virus by muscle injection but not oral delivery of bacterially expressed dsRNAs. *Dis. Aquat. Org.* 95: 19–30.

Tian JY, Sun XQ, Chen XG. 2008. Formation and oral administration of alginate microspheres loaded with pDNA coding for lymphocystis disease virus (LCDV) to Japanese flounder. Fish Shellfish Immunol 24: 592-599.

Treerattrakool S, Panyim S, Udomkit A. 2011. Induction of ovarian maturation and spawning in *Penaeus monodon* broodstock by double-stranded RNA. *Mar. Biotechnol* 13: 163–169.

Treerattrakool S, Chartthai C, Phromma-in N, Panyim S, Udomkit, A. 2013. Silencing of gonad-inhibiting hormone gene expression in *Penaeus monodon* by feeding with GIH dsRNA enriched *Artemia*. *Aquaculture* 404: 116–121.

Ventura T, Manor R, Aflalo ED, Weil S, Raviv S, Glazer Sagi, A. 2009. Temporal silencing of an androgenic gland-specific insulin-like gene affecting phenotypical gender differences and spermatogenesis. *Endocrinology* 150: 1278–1286.

Witteveldt J, Vlak JM, and van Hulten MCW. 2004. Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. *J. Virol.*, 78; 2057-2061.

Xu, J., Han, F., Zhang, X. 2007. Silencing shrimp white spot syndrome virus (WSSV) genes by siRNA. *Antivir. Res.* 73: 126–131.

Acute Toxicity of Garlic (*Allium sativum*) Extract to Snubnose Pompano (*Trachinotus blochii*) Juvenile

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Abstract

Garlic (*Allium sativum*) is a well-known medicinal herb which has been shown to possess anti-microbial and anti-parasitic properties. This study was conducted to test the toxicity levels of snubnose pompano (*Trachinotus blochii*) juvenile to garlic (*Allium sativum*) extract by determining the cumulative mortality and median lethal concentration (LC50). Test fish were exposed to six concentrations of the extract (2.5, 5.0, 7.5, 10, 20 and 30 ppm) in a 96-hour static bioassay. Cumulative mortality was highest at 100 % for 30 ppm garlic extract, with mortalities found to increase with increasing concentration. Test fish exposed to 20 and 30 ppm exhibited weak and static behavior. The LC50 of garlic extract to *T. blochii* was found to be 7.48 ppm at 96 h. Findings of the present study suggest that aqueous garlic extract up to 5 ppm can be safely used in pompano for prophylactic purposes.

Keywords: Median lethal concentration (LC50), garlic extract, pompano

Introduction

Aquaculture has emerged as the fastest growing food-producing sector in the past few decades (FAO, 2014) but has been affected with the emergence of parasitic diseases (Palma *et al*., 2015; Akoll *et al*., 2012; Nowak, 2007). Numerous anti-parasitic and chemical treatments have been used to control fish parasites. Although these existing therapeutants are effective against ectoparasites, herbal or natural extracts are an environment-friendly alternative. There have been numerous reviews about the potential use of medicinal herbal extracts in aquaculture (Bulfon *et al*., 2013; Reverter *et al*., 2014; Van Hai, 2015). Moreover, the effectiveness of plant-derived substances in controlling fish parasites have been widely studied (Picon-Camacho *et al*., 2012; Erguig *et al*., 2015; Wunderlich *et al*., 2017; Tavares-Dias, 2018). Garlic (*Allium sativum*) is one of the herbal remedies that help in the control of fish pathogens. Allicin has been identified as the major active pharmaceutical molecule found in crushed garlic. It is produced when the chemical compound alliin is catalyzed by the enzyme allinase which happens when the garlic bulb is crushed, sliced or processed mechanically (Tracy and Kingston, 2007).

It has been reported that garlic extract has antihelminthic properties against *Capillaria* sp. in the common carp (*Cyprinus carpio*) (Peña *et al*., 1988), inhibitory effects for fish parasite infestation against *Ichthyopthirius multifiliis* (Buchmann *et al*., 2003) in Nile tilapia (*Oreochromis niloticus*), trichodiniasis in eel (*Anguilla anguilla*) (Madsen *et al*., 2000), *Neobenedenia* sp. (Militz *et al*., 2013) in farmed barramundi (*Lates calcarifer*) and treatment of *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) (Fridman *et al*., 2014).

Snubnose pompano (*T. blochii*), is considered a potential marine fish species for aquaculture because of its fast growth rate, excellent meat quality, and suitability for cage culture (Ma *et al*., 2014). Currently, sea lice infestation belonging to family Caligidae is the most significant disease problem affecting pompano, *T. blochii* reared in cages and tanks (Pakingking *et al*., 2018; Reyes *et al*., 2014; Cruz-Lacierda *et al*., 2011). In a study by Cruz-Lacierda *et al*. (2011), parasitic caligid identified as *Lepeophtheirus spinifer* caused lesions on the body surface and heavy infestation can cause high or mass mortality of *T. blochii*.

There have been growing concerns regarding the use of chemicals in parasite control due to reports of reduced efficacy and its effect on inducing resistance to fish pathogens when used inappropriately (Lee and Gao, 2012; Yanong, 2008). Malachite green and formalin are examples of conventional treatments for parasite infestation but can possibly be more toxic to the hosts rather than their parasites (Schelkle *et al*., 2013). An alternative way to solve this problem is to use medicinal or herbal plants like garlic (*A. sativum*). There are many reports about the antiparasitic activities of garlic in fish species (Ankri and Mirelman, 1999; Madsen *et al*. 2000; Buchmann *et al*., 2003). However, none of these previous studies have evaluated the efficacy of garlic extract in terms of the acute toxicity of pompano. Thus, the present study was conducted to determine the 96 h median lethal concentration (LC50) of aqueous garlic extract for pompano juveniles.

Materials and methods

Experimental set-up

Pompano juvenile (BW=138.8±41.1 g, TL=19.5±1.51 cm) obtained from Marine Hatchery (SEAFDEC/AQD), Tigbauan, Iloilo, Philippines were acclimatized for at least 2 weeks under laboratory conditions (salinity = 30 ppt, temperature = 280C). Fish were placed in a 500-L fiberglass tank provided with flow-through seawater system and gentle aeration. Fish were fed daily at 3 % body weight with feed for pompano formulated by SEAFDEC/AQD until use for the experiment.

Preparation of aqueous garlic extract

A 3 L stock solution at 1000 ppm garlic extract was prepared by dissolving 3 g of powdered garlic extract (Hebei Kangdali Pharmaceutical Co., Ltd., 25 % allicin) in 3 L distilled water. All concentrations were set using the formula: M1V1=M2V2.

Acute toxicity (LC_{50}) experiment

Two trials each of 96 h static bioassay tests were conducted at the Fish Health Wet Laboratory of SEAFDEC following standard procedures for toxicity tests outlined by the APHA-AWWA-WEF (2012). A completely randomized design was used for experiments with 10 fish per 20 L of seawater in glass aquaria provided with aeration. After a 1 hr acclimatization period (28 °C; 32 ppt salinity; 7.8 pH), six test concentrations were prepared (2.5, 5.0, 7.5, 10, 20 and 30 ppm) by adding the necessary volume of stock solution to the

aquaria (**Table 1**). For the control, only seawater was used. All test concentrations including the control were done in three replicates per trial. Test fish were not fed during the entire experimental period.

Behavioral patterns of fish

Fish were observed every 24 h for four days (96 h) for behavioral changes and mortality. Moribund fish were removed and dissected immediately. Gills and liver of moribund fish were collected for histological analysis. Dead fish were removed, recorded and properly disposed. Water quality monitoring

Water quality parameters, i.e. salinity, temperature, dissolved oxygen, were measured and recorded daily. Temperature and salinity were measured using standard mercury thermometer and an AtagoS/ Mill hand-held optical refractometer, respectively. Dissolved oxygen and pH were measured using digital Milwaukee MW 600 DO meter and Milwaukee MW 101 pH meter, respectively.

Histological analysis

The gills and liver samples from the control fish and exposed fish were subjected to histological processing. The gills and liver of *T. blochii* were dissected and fixed in Bouin's fixative. Tissues were processed, sectioned and stained with haematoxylin and eosin (Humason, 1979). Stained tissue samples were examined under a compound microscope.

Statistical analysis

Probit analysis program by Srinivasan (2004) based on Probit Analysis by Finney (1971) was used to calculate the median lethal concentration (LC50) values and corresponding 95 % confidence limits.

Results

Table 2 shows the values of water parameters measured during the 96 h of the experiment. The average temperature (28±0.0), salinity (32 ppt) and pH (8.0) were consistent until the last day of monitoring. However, the mean dissolved oxygen level decreased towards 96 h but the level was above 5 ppm.

In terms of behavior, test fish immersed in 10 to 30 ppm extract exhibited abnormal behavior in the first 24 h of the experiment. Fish became almost static upon addition of the said garlic extract concentrations to their respective aquaria. Test fish exposed to highest concentration (30 ppm) remained weak throughout the first 24 h, with frequent surface-to-bottom swimming and faster opercula activity were observed.

Table 1. Volume of stock solution added to experimental water for the different test concentration

Test concentrations (ppm)	Volume (ml) of stock solution (1000 ppm)
2.5	50
5	100
7.5	150
10	200
20	400
30	600

Table 2. Water parameter values (mean±SD) measured during the 96 h exposure of *T. blochii* to garlic extract (Hebei Kangdali Pharmaceutical C. Ltd., 25 % allicin) concentration

Time (h)	Temperature (^{0}C)	Salinity (ppt)	pH	Dissolved oxygen (ppm)
0	28±0.0	32±0.0	8.04±0.01	9.0±0.2
24	28±0.0	32±0.0	7.12±0.02	8.66±0.2
48	28±0.0	32±0.0	7.86±0.02	6.6±0.2
72	28±0.0	32±0.0	7.83±0.02	6.0±0.2
96	28±0.0	32±0.0	7.83±0.02	5.0±0.3

Median lethal concentration or LC50 is an aqueous chemical activity which causes 50% mortality in the exposed population of fish. The 96 h LC50 test was conducted to measure the susceptibility and survival potential of fishes to a particular toxic substance. Higher LC50 value indicates lower toxicity because greater concentrations are required to cause 50% mortality in fishes. The cumulative mortality of snubnose pompano after exposure to garlic extract for 96 h was shown in **Figure 1**. The cumulative mortality rates were 0, 23.33 %, 53.33 %, 73.33 %, 86.66 % and 100 % at the concentrations of 2.5, 5.0, 7.5, 10, 20 and 30 ppm, respectively. No mortality was recorded in the control group. The 96 h LC50 was 7.48 ppm with lower and upper confidence limits of 6.19 ppm and 9.04 ppm respectively (**Table 3**).

Histopathological changes in the gills and liver were observed in 7.5, 10, 20 and 30 ppm. **Figure 2** shows the gills of pompano from the control group. At lower concentrations (2.5 and 5 ppm), gills appeared to be normal (**Figure 3**). Slight epithelial lifting and moderate hyperplasia occurred in fish treated with 10 and 7.5 ppm garlic extract after 96 h (**Figure 4**). Gill damage such as significant number of epithelial lifting, severe hyperplasia and fusion of the lamellae were noted in fish exposed to 20 ppm at 96 h and 30 ppm at 48 h (**Figure 5**). **Figure 6** shows liver cell morphology of the control group. Histopathological changes were not observed in lower concentrations (2.5 ppm and 5 ppm) (**Figure 7**). Fish exposed to 7.5 ppm and 10 ppm exhibited slight hypertrophy of the vacuolated-hepatocytes and sinusoidal dilation were also observed (**Figure 8**). Moreover, the frequency and intensity of change at 96 h of exposure was less than that of fish exposed

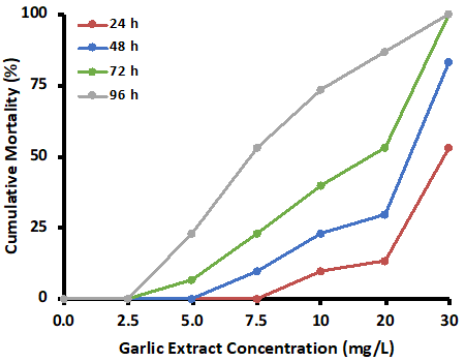

Figure 1. Percentage cumulative mortality of *T. blochii* exposed to different concentrations of garlic extract for various exposure time

Table 3. Computed lethal concentration (LC) values and confidence limits after 96h exposure of *T. blochii* juveniles (TL=19.5±1.51 cm) to garlic extract using Probit analysis.

Point	Probit	Concentration (ppm)	Log Concentration	95 % Confidence Limits	
				Lower	Upper
LC_1	2.67	0.60	-0.22	0.25	1.42
LC_{10}	3.72	1.65	0.22	0.95	2.86
LC_{50}	5.00	7.48	0.87	6.19	9.04
LC_{95}	6.64	28.43	1.45	17.78	45.47
LC_{99}	7.33	55.13	1.74	28.50	106.64

to higher concentrations (20 and 30 ppm). Blood congestion with hypertrophied vacuolated-hepatocytes, sinusoidal dilation and pyknosis were observed in 20 ppm at 96 h and 30 ppm garlic extract at 48 h (**Figure 9**).

Discussion

Measured temperatures were within the optimum range for *T. blochii*, which is 24-280C and optimum temperature is 27 °C (FAO, 2016). Salinity was also constant at 32 ppt throughout the experiment.

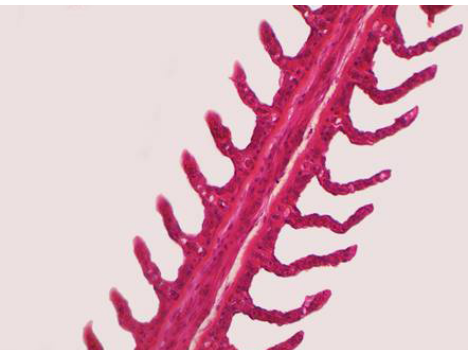

Figure 2. Section through the gills of pompano (*T. blochii*) of control group having no histopathological changes at 96 h. (H&E, 40x)

Salinity and pH values measured are within the optimum range for *T. blochii*, which are 5-40 ppt for salinity and 7.5-8.5 for pH (Jayakumar *et al*., 2013). According to Jayakumar *et al*. (2013), dissolved oxygen level for pompano should be maintained above 5 ppm.

T. blochii are highly active fishes and very sensitive to stimuli such as movements according to Reyes *et al*. (2014) thus it is considered an abnormal behavior for test fish to be static. Results pertaining to changes in behavior of *T. blochii* also agree with the findings of Syngai *et al*. (2016) wherein they also observed the carps gulping for air and having a static or slow swimming behavior after the addition of garlic aqueous solutions in the aquaria. In the study of Aqel *et al*. (1991), the garlic juice was tested *in vitro* to the isolated segments of aorta, trachea, intestine and heart of a rabbit. Their results imply that garlic juice has a relaxant effect on the smooth muscles of the rabbit. Acetylcholine is a neurotransmitter responsible for the action of muscles and it was inhibited by the addition of garlic juice. This evidence may be associated with the slow action and swimming behavior of test fish.

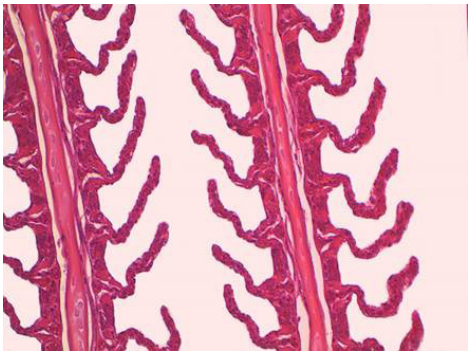

Figure 3. Section through the gills of pompano (*T. blochii*) exposed to 5 ppm having no histopathological changes at 96 h. (H&E, 40x)

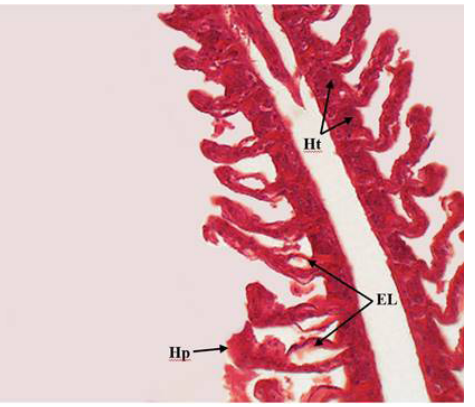

Figure 4. Section through the gills of pompano (*T. blochii*) exposed to 7.5 ppm garlic extract at 96 h showing slight epithelial lifting, moderate hyperplasia and hypertrophy. (H&E, 40x). EL=epithelial lifting, Hp=hyperplasia, Ht=hypertrophy

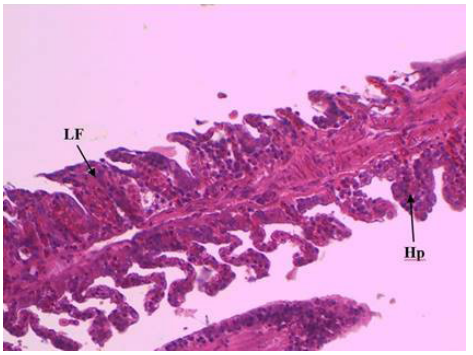

Figure 5. Section through the gills of pompano (*T. blochii*) exposed to 30 ppm garlic extract at 48 h showing severe hyperplasia and lamellar fusion of the secondary lamellae. (H&E, 40x). Hp=hyperplasia, LF=lamellar fusion

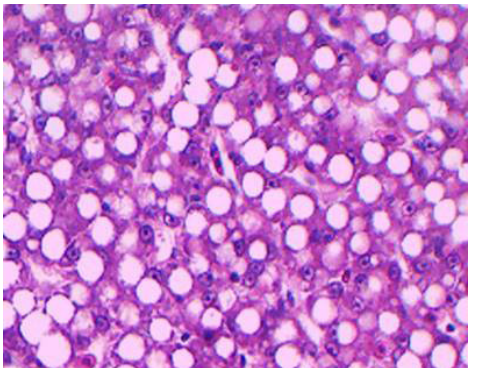

Figure 6. Section through the liver of pompano (*T. blochii*) of control group having no histopathological changes at 96 h. (H&E, 100x)

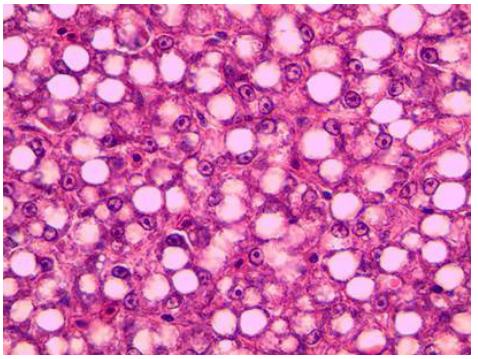

Figure 7. Section through the liver of pompano (*T. blochii*) exposed to 5 ppm having no histopathological changes at 96 h. (H&E, 100x)

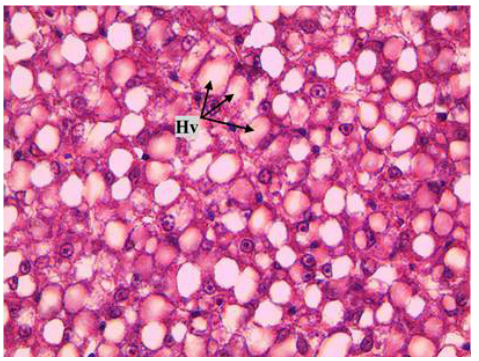

Figure 8. Section through the liver of pompano (*T. blochii*) exposed to 7.5 ppm garlic extract at 96 h showing slightly hypertrophied hepatocytes. (H&E, 100x). Hv=hypertrophied vacuolated hepatocytes

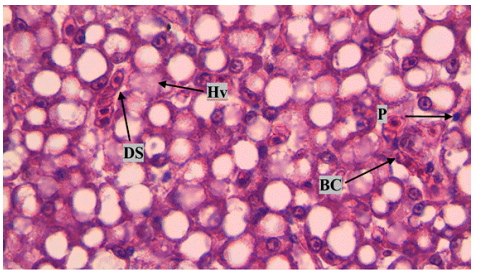

Figure 9. Section through the liver of pompano (*T. blochii*) exposed to 30 ppm garlic extract at 48 h showing blood congestion, dilated sinusoid, hypertrophied hepatocytes and pyknotic nuclei in some hepatocytes. (H&E, 100x). BC = blood congestion, DS = Dilated sinusoid, Hv = hypertrophied vacuolated hepatocytes, P = pyknosis.

Flores-Lopes and Thomaz (2011) reported that physiological responses of fish gills to irritants in water include inflammation, hyperplasia, lamellar fusion and epithelial lifting of the secondary lamellae. Similarly, *T. blochii* exposed to 7.5 to 30 ppm garlic extract showed varying degrees of epithelial lifting, hyperplasia and fusion of the secondary lamellae (Fig. 2). Epithelial lifting is one of the first pathological responses of the gills to pollutants by increasing the distance between the water and blood to block the entry of contaminants (Flores-Lopez & Thomaz, 2011). Lamellar fusion is also a defense mechanism of the fish against pollutants but limits gas exchange (Dane & Sisman, 2014).

Blood congestion, pyknosis, hypertrophied hepatocytes and sinusoidal dilation observed in the liver of *T. blochii* exposed to 7.5 to 30 ppm garlic extract are stress responses of fish to accumulation of toxicants (Kroemer *et al*., 2008). These histopathological changes were also recorded in the study of Al-Salahy & Mahmoud (2003) where they orally administered the garlic juice in Nile tilapia for 5 days and 11 days every 24 h and among the changes, cellular degeneration was the most abundant. Moreover, this is an indication that higher concentrations of garlic extract can induce histopathological changes in pompano. Supposedly, a normal hepatocyte is polygonal in shape with a centrally-located spherical nucleus. Vacuolation of the hepatocytes may be associated with high lipid diet of the fish prior to the experimental period. Hepatocytes oxidize fatty acids, however, in large quantities, hepatocytes are overwhelmed thus unable to oxidize the excess, causing fat deposition to occur. In the study of Fountoulaki *et al*. (2017), the lipid deposition in hepatocytes increased in meagre fish when fed with 20% lipid feeds. They observed steatosis or abnormal fat accumulation manifested by the vacuolation of hepocytes. However, Fountoulaki *et al*. (2017) implied that vacuolation of hepatocytes has no pathological consequences as the growth of test fish were consistently increasing over time. These changes also manifested in the liver of *Tor tambroides* fed with dietary linoleic and linoleic acid ratio where intense accumulation of lipid manifested as well (Ramezani-Fard *et al*., 2011). However, the pathological effects steatosis are not well studied. This fatty liver condition possibly is a result of an imbalanced diet and may be irreversible (Fountoulaki *et al*., 2017).

Fujisawa *et al*. (2008) have stated that allicin has low solubility in polar solvents like water, which may suggest that undissolved residues may have accumulated in the gills of *T. blochii* and reduced ionic and gas exchanges, thereby affecting its breathing. In the present study, water was also used as solvent for garlic extract because of the stability and longer chemical half-life of the active ingredient of garlic in water (Fujisawa *et al*., 2008). Similar findings due to accumulation of excess extracts were stated in a study by Claudiano *et al*. (2012), in which guppy (*Phalloceros caudimaculatus*) exposed to *Terminalia cattappa* extract showed difficulty in breathing. This further supports the possibility that the difficulty in breathing manifested in the surface-to-bottom swimming of *T. blochii* in the present study was due to the accumulation of undissolved garlic extract in gills.

Results in terms of mortality is in agreement with the study of Syngai *et al*. (2016) in common carp juveniles immersed in allicin where mortality rate also increased with increasing concentration of garlic extract. The present results further support the claim that lethality and susceptibility of *T. blochii* to garlic extract is concentration-dependent, as mortalities increased with increasing garlic extract concentration.

Conclusion

The findings of the present study suggest that aqueous garlic extract up to 5 ppm can be safely used in pompano for prophylactic purposes. The acute toxicity data obtained from this study provide baseline information on the dosage for the aqueous garlic extract to be used for future prophylaxis. *In vitro* and *in vivo* studies particularly on the efficacy of garlic extract in controlling and preventing parasitic diseases in various fishes are needed.

Acknowledgment

The author expresses her gratitude to the Government of Japan Trust Fund (GoJ-TF6 (8400-T-RD-FH0215) for funding this work. Thanks are due to Ms. Haydee Dumaran-Paciente and Mr. Elvis Biñas for their technical assistance.

References

Akoll P, Koneeny R, Mwanja W W, Nattabi K J, Agoe C, and Schiemer F. 2012. Parasite fauna of farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) in Uganda. *Parasitol Res*. 110: 315-323.

Al-Salahy M and Mahmoud A A B. 2003. Metabolic and histological studies on the effect of garlic administration on the carnivorous fish *Chrysichthys auratus*. *Egypt J Biol*. 5: 94-107.

Ankri S and Mirelman D. 1999. Antimicrobial properties of allicin from garlic. *Microbes and Infection* 2: 125-129.

APHA, AWWA, WEF. Standard methods for examination of water and wastewater 22nd ed. 2012. Washington: American Public Health Association. 1360 pp.

Aqel M, Gharaibah M, and Salhab A. 1991. Direct relaxant effects of garlic juice on smooth and cardiac muscles. *Journal of Ethnopharmacology*. 33(1–2): 13–19.

Buchmann K, Jansen P B, Kruse K D. 2003. Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* thereonts and tomonts *in vitro* experiments. *North American J. of Aquaculture* 65: 21-24.

Bulfon C, Yolpatti D and Galeoti M. 2015. Current research on the use of plant derived products in farmed fish. *Aqua. Res*. 46: 513-551.

Claudiano G S, Pilarski F, Cruz C, Salvador R, Belo M A and Moares M R. 2012. Lethal concentration of aqueous extract of leaves of *Terminalia catappa* in guppy *Phalloceros caudimaculatus*. Archives of Veterinary Science 17: 15-19.

Cruz-Lacierda, ER, Pagador GE, Yamamoto A and Nagasawa K. 2011. Parasitic caligid copepods of farmed marine fishes in the Philippines, pp. 53-62. In Bondad-Reantaso MG, Jones, J B Corsin, F and Aoki T (eds.). Diseases in Asian Aquaculture VII. Fish Health Section, Asian Fisheries Society, Selangor, Malaysia. 385 pp.

Dane H and Sisman T. 2014. Histopathological changes in gill and liver of *Capoeta capoeta* living in the Karasu River, Erzurum. *Environmental Toxicology*. 30(8): 904–917.

Erguig M, Yahyaoui A, Fekhaoui M and Dakki M. 2015. The use of garlic in aquaculture. *European J of Biotech Biosci*. 3: 28–33.

FAO (Food and Agriculture Organization). 2014. Fisheries and Aquaculture Information and Statistics Service, Aquaculture production: quantities. 1950–2014. FISHSTAT Plus Universal software for fishery statistical time series.

FAO (Food and Agriculture Organization). 2016. Cultured aquatic species information programme *Trachinotus* spp. FAO http://www.fao.org/fishery/cuktured species/*Trachinotus*_spp/en

Flores-Lopes F and Thomaz A. 2011. Histopathologic alterations observed in fish gills as a tool in environmental monitoring. *Brazilian Journal of Biology*. 71(1): 179–188.

Finney D J. 1971. Probit analysis (Third edition). New York, Wiley Interscience. 333 pp.

Fountoulaki E, Grigorakis K, Kounna C, Rigos G, Papandroulakis N, Diakogeorgakis J, and Kokou F. 2017. Growth performance and product quality of meagre (*Argyrosomus regius*) fed diets of different protein/lipid levels at industrial scale. *Italian Journal of Animal Science*. 16(4): 685–694.

Fridman S, Sinai T, Zilberg D. 2014. Efficacy of garlic based treatments against monogenean parasites infecting the guppy (*Poeilia reticulate* (Peters), *Vet. Para.* 203: 51-58.

Fujisawa H, Sinai K, Origuchi K, Kumagai H, Seki T, Ariga T. 2008. Biological and chemical stability of garlic-derived allicin. *J. of Agri. And Food Chem.* 56: 4229-4235.

Humason G L. 1979. Animal Tissue Techniques (4th ed.). W H Freeman & Co

Jayakumar R, Abdul Nazar AK and Gopakumar G. 2013. Culture of silver pompano *Trachinotus blochii* in coastal aquaculture ponds. CMFRI Open Accesss Institutional Repository. http://eprints.cmfri.org. in/9725/

Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri E S, Baehrecke, E H, Blagosklonny M V, El-Deiry W S, Golstein P, Green D R, Hengartner M, Knight R A, Kumar S, Lipton S A, Malorni W, Nuñez G, Peter M E, Tschopp J, Yuan J, . . . Melino G. 2008. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death & Differentiation*. 16(1): 3–11.

Lee J Y and Gao Y. 2012. Review of the application of garlic *Allium sativum* in aquaculture. *J. of the World Aquac. Soc*. 43:447-458.

Ma Z, Guo H, Zheng P, Wang L, Jiang S, Qin JG and Zhang, D. 2014. Ontogenetic development of digestive functionality in golden pompano *Trachinotus ovatus* (Linnaeus 1758). *Fish Physiol Biochem.* 40:1157–1163.

Madsen H C K, Buchmann K and Mellergaard S. 2000. Treatment of trichodiniasis in eel (*Anguilla anguilla*) reared in recirculation systems in Denmark: alternatives to formaldehyde. Aquaculture. 186: 221-231.

Martins M L, Moraes F R, Miyazaki D M Y, Brum C D, Onaka E M, Fenerick J, Jr., and Bozzo F R. 2002. Alternative treatment for *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) infection in cultivated pacu *Piaractus mesopotamicus* (Osteichthyes: Characidae) in Brazil and their haematological effects. Parasite. 9: 175–180.

Militz F, Thane A, Southgate P C, Carton A G and Hutson K S. 2013. Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. Aquaculture 408: 95–99.

Nowak B F. 2007. Parasitic diseases in marine cage culture: an example of experimental evolution of parasites? *Int. J. Parasitol*. 37: 581-588.

Pakingking Jr. R, Bautista N B, Catedral D, de Jesus-Ayson EG. 2018. Characterisation of *Vibrio* isolates recovered from the eyes of cage-cultured pompano (*Trachinotus blochii*) infested with caligid parasites (*Lepeophtheirus spinifer*). *European Association of Fish Pathologists Bulletin*. 38(1): 35-41.

Palma P, Cruz-Lacierda E and Corre V. 2015. The use of potassium permanganate (KMnO4) against trichodiniases on milkfish (*Chanos chanos*) fingerlings. *Bulletin of the European Association of Fish Pathologists*. 35: 201-207.

Peña N, Auro A and Sumano HA. 1988. A comparative trial of garlic, its extract and ammonium potassium tartrate as anthelmintics in carp. *J. Ethnopharmacology*. 24: 199-203.

Picon-Camacho S M, Marcos-Lopez M, Bron J E, Shinn AP. 2012. An assessment of the use of drug and non-drug interventions in the treatment of *Ichthyophthirius multifiliis* Fouquet, 1876, a protozoan parasite of freshwater fish. *Parasitol*. 139:149–190.

Ramezani-Fard E, Kamarudin M S, Ehteshami F, Zadeh S, Saad C and Zokaeifar H. 2014. Effect of dietary linolenic acid (18:3n-3)/linoleic acid (18:2n-6) ratio on growth performance, tissue fatty acid profile and histological alterations in the liver of juvenile *Tor tambroides*. Iranian Journal of Fisheries Sciences. 13:185-200.

Reverter M, Bontemps N, Lecchini D , Banaigs B and Sasal P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture. 433:50-61.

Reyes O S, de Jesus-Ayson E G, Pedroso F and Cabanilla MI. 2014. Hatchery production of snubnose pompano *Trachinotus blochii* Lacepede. Aquaculture Extension Manual No. 56. SEAFDEC Aquaculture Department,Tigbauan, Iloilo, Philippines. 25 pp.

Schelkle B, Snellgrove D and Cable J. 2013. *In vitro* and *in vivo* efficacy of garlic compounds against *Gyrodactylus turnbulli* infecting the guppy (*Poecilia reticulata*). *Veterinary Parasitology*. 198(1–2): 96–101.

Srinivasan MR. 2004. Probit Analysis. *In:* Palaniswamy S, Kuttalam S, Chandrsassekaran S, Kennedy JS and Srinivasan MR, editors. Electronic Manual on Pesticides and Environment. Coimbatore, India. Department of Agricultural Entomology Tamil Nadu Agricultural University. 745 pp.

Syngai G G, Dey S and Bharali R. 2016. Evaluation of toxicity levels of the aqueous extract of *Allium sativum* and its effects on the behavior of juvenile common carp (*Cyprinus carpio*) L., 1758). *Asian J Pharm Clin Res*. 9: 417-421.

Tavares-Dias M. 2018. Current knowledge on use of essential oils as alternative treatment against fish parasites. *Aquat Living Resour*. 31: 13.

Tracy T S and Kingston RL. 2007. *Herbal Products: Toxicology and Clinical Pharmacology (Forensic Science and Medicine)* (2nd ed.). Humana. 125-127 pp.

Van Hai H. 2015. The use of medicinal plants as immunostimulants in aquaculture: a review. *Aquaculture*. 446: 88-96.

Wunderlich A C, Guimaraes A C and Takeara R. 2017. Plant-derived compounds as an alternative treatment against parasites in fish farming: a review. In Khater H, Govindarajan M, Benelli G, editors. Natural remedies fight against parasites. IntechOpen, p. 246.

Yanong R P E. 2008. Use of hydrogen peroxide in finfish aquaculture. University of Florida IFAS Extension. https://agrilifeedn. tamu.edu/fisheries/files/2013/09/Use-of-Hydrogen-Peroxide-in-Finfish-Aquqculture.pdf. Accessed 2019 May 5.

Factors Affecting Mortality of Shrimp, *Penaeus monodon*, Experimentally Infected With *Vibrio parahaemolyticus* Causing Acute Hepatopancreatic Necrosis Disease (VP_{AHPND})

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Abstract

One of the most recent diseases affecting the shrimp industry is the early mortality syndrome (EMS). EMS, characterized by observed mortality in shrimp within the first 35 days of culture, is due to several diseases, one of which is the acute hepatopancreatic disease (AHPND). Outbreaks due to AHPND have caused economic losses to many shrimp producing countries globally. This paper investigates factors affecting mortality of shrimp, *Penaeus monodon* experimentally infected with *Vibrio parahaemolyticus* causing AHPND (VP_{AHPND}).

Tank experiments done suggested that exposure to 10^7 cfu/ml VP_{AHPND}, 35 °C, and 10 and 28 ppt increase the risk of shrimp mortality due to AHPND. The VP_{AHPND} concentration in the water that *P. monodon* can overcome is <105 cfu/ml. Observed mortality due to VP_{AHPND} is age related, with higher mortalities in younger infected shrimp.

Introduction

One of the most recent diseases affecting the shrimp industry is the early mortality syndrome (EMS). EMS is characterized by observed mortality in shrimp within the first 35 days of culture. It is due to several diseases, one of which is the acute hepatopancreatic necrosis disease (AHPND) caused by a virulent strain of *Vibrio parahaemolyticus* (Flegel, 2016). AHPND has been reported in China, India. Malaysia, Mexico, Philippines, Thailand and Viet nam (Flegel, 2012; Lightner et. al, 2012; Soto-Rodriguez *et al*., 2015; De la Pena *et al*., 2015). AHPND is caused by a unique strain of *Vibrio parahaemolyticus* (VP_{AHPND}) that has transferrable plasmid carrying the PU:AB-like toxin genes (Tran *et al*., 2013). Variation in *V. parahaemolyticus* isolates from shrimp experiencing AHPND outbreak has been reported (Joshi *et al*., 2014; Kumar *et al*., 2014; Kondo *et al*., 2014).

Outbreaks due to AHPND have caused economic losses to many shrimp producing countries globally. Mortalities due to AHPND have been reported to occur 10–30 days after stocking of post larvae (PL) in ponds (Joshi *et al*., 2014b; Leaño and Mohan, 2012; Soto-Rodriguez *et al*., 2015). The disease can cause up to 100 % cumulative mortality in affected PL within

a week. However, in older penaeids at DOC 46 and 96, lower mortalities of 40–60 % can be observed (de la Peña *et al*., 2015).

Several reports have identified farm level risk factors. Some of these are high salinity or salinity below 5 ppt; high and fluctuating temperature; and pH>7 (FAO, 2013; Bondad-Reantaso and Arthurs, 2018). Salinity of 20 ppt reduces disease incidence (http://www.agriculture.gov.au/pests-diseases-weeds/aquatic).

Infection studies were done to determine and understand the pathogencity of the VP_{AHPND} to healthy shrimp. Shrimp are infected by immersing them in high concentrations of VP_{AHPND} at high stocking density for 15 min and cultured in water with different concentrations of the bacteria (Tran *et al*., 2013; Lai, 2015). This method stresses the shrimp and is far from natural infection. In this study we investigated the VP_{AHPND} concentration in the water that can cause mortality in healthy *P. monodon* without subjecting them to stress, thus simulating natural infection. We also investigated two reported risk factors, salinity and temperature. Age related infection was also verified.

Materials and methods

Experimental shrimp

P. monodon (PL 20) were purchased from a local hatchery and stocked in a concrete tank with seawater and provided with aeration. Shrimp were fed with commercial pellet at 4 % body weight given twice daily until use. Shrimp were starved a day prior to the experiment. Shrimp were analyzed and tested negative using PCR for known shrimp viruses (white spot syndrome virus (WSSV), taura syndrome virus (TSV), infectious hypodermal hemapoietic necrosis virus (IHHNV), yellowhead virus (YHV), infectious myonecrosis virus (IMNV), monodonbaculovirus (MBV) and hepatopancreatic virus (HPV)) and AHPND before the pathogenicity/challenge experiments.

Bacterial isolate

Vibrio parahaemolyticus used in the experiment were from AHPND cases in the Philippines and confirmed to be the AHPND bacteria (dela Pena *et al*., 2015). The bacterium was sub-cultured in *Vibrio* Chromogenic Agar (VCA, Pronadisa) and harvested after 18–24 hours' incubation. Harvested bacteria were suspended in tryptic soy broth with 2 % NaCl added (TSB+, Merck). The bacterial count of the inoculum was confirmed by plating onto VCA; presence of AHPND was confirmed by PCR.

Investigation of factors affecting mortality in VP_{AHPND} infected shrimp

Effect of bacterial concentration and exposure method

Glass aquaria, 10 L capacity, were used for the experiment. Clean aquaria (washed with detergent and water) were sterilized by swabbing with cotton soaked in alcohol and covered with sterile-aluminum foil wrapped plywood. Aquaria were filled with 5 L of UV sterilized seawater, and provided with aeration. Plastic tubings, air stones, connectors and other paraphernalia were sterilized by pouring with boiled water prior to use.

Two infection methods were investigated using healthy *P. monodon* post larvae (PL): unimmersed and pre-immersed. In both methods, *P. monodon* PL (ABW=0.18+ 0.04 g) were stocked at 15 ind/aquaria. Three replicates were carried out for each concentration and control group.

Unimmersed. For this method, 21 aquaria were prepared as previously described and stocked with healthy shrimp. One hour after stocking, the prepared bacterial suspension was added into the treated aquaria to obtain the desired concentrations of 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 cfu/ml. Sterile TSB+ was added onto aquaria that served as the control.

Pre-immersed 1. For this method, healthy *P. monodon* PL were immersed in VP_{AHPND} 10^7 cfu/ml bacterial solution at 1 ind/ml for 15 min prior to stocking in 21 aquaria, prepared as previously described. One hour after stocking, the prepared bacterial suspension was added into the aquaria to obtain the desired concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 cfu/ml. Sterile TSB+ was added onto aquaria that served as the positive control.

Pre-immersed 2. This was done to confirm the results obtained in pre-immersed 1, wherein there is no significant difference in the mortality rate observed in all treatments including the control. The same methodology was followed except that two controls were used: positive and negative; and lower final bacterial concentrations of 10^2, $10^{3,}$ 10^4 cfu/ml were used to inoculate the aquaria. A total of 15 aquaria were prepared as previously described. Nine of the aquaria were inoculated with the VP_{AHPND} bacterial suspension to give lower final concentrations of 10^2, 10^3, 10^4 cfu/ml done in triplicates. PL were pre-immersed in 10^7 cfu/ml VP_{AHPND} bacterial solution at 1 ind/ml for 15 min prior and stocked in the 9 VP_{AHPND} inoculated aquaria and in 3 aquaria inoculated with sterile TSB+ which served as the positive control. For the negative control, *P. monodon* PL were immersed in UV sterilized seawater at 1 ind/ml for 15 min prior to stocking in three aquaria with UV sterilized seawater with TSB+ added; no bacteria were added into the aquaria. The experiments were terminated after 120 h.

Monitoring

Shrimp mortality was monitored daily. Samples were taken at 24, 72, 120 hpi for bacteriology and AHPND detection by PCR. For bacteriology, shrimp were rinsed 3x in sterile seawater and suspended in TSB for 1 h. After the 1 h incubation, the shrimp was homogenized and serially diluted. Representative serial dilutions were plated onto *Vibrio* chromogenic agar (VCA) for the presumptive *Vibrio parahaemolyticus* counts. Inoculated NA and TCBS plates were incubated for 24 h; VCA plates for 48 h.

Water samples were taken before stocking with shrimp, after adding bacteria and daily thereafter. Samples were serially diluted and plated onto VCA.

Effect of salinity and temperature

Fiberglass tanks, 120 L capacity filled with 50 L UV sterilized water were used in the experiment. Tanks were provided with aeration. Plastic tubing, air stones, connectors and other paraphernalia were sterilized by pouring with boiled water prior to use.

Tanks were stocked with *P. monodon* PL (ABW= 0.31+0.06) at 25 ind/aquaria. One day after stocking, salinity and temperature were gradually adjusted to the desired level. Water heater were used to increase water temperature. Ambient UV sterilized seawater was diluted with cartridge filtered freshwater in a flow through system to lower the salinity.

The salinity used were 10, 20 and 28 ppt at ambient temperature. Temperature used were 31 °C and 35 °C at 20 ppt salinity. Two sets of tanks for each treatment in triplicate were prepared. One set for uninfected and the other set, for infected. Desired salinity and temperature levels were attained after 3–4 days. One day

after attaining the desired salinity and temperature, shrimp were removed from each tank, one tank at a time. For the infected group, shrimp were immersed in 10^7cfu/ml VP_{AHPND} bacterial suspension at 1 shrimp/ml for 15 min. Uninfected group were immersed in UV sterilized seawater at 1 shrimp/ml for 15 min. After immersion, shrimp were returned to the tanks were they were originally stocked or were taken. The experiment was terminated after 120 h.

Effect of age

Glass aquaria (10 in x 6 in x 12 in) covered with sterile-aluminum foil wrapped plywood were used in the experiment. The aquaria were filled with 5 L UV sterilized seawater and provided with aeration.

Shrimp used in this study were from the same batch cultured in concrete tanks to attain the desired days of culture. The age/size used were 0.14 g at PL 27 and 1.3 g at PL 150. In this test, shrimp were immersed in 10^7 cfu/ml VP_{AHPND} bacterial suspension for 15 min at 1 shrimp/ml and cultured (15 PL/5L) in UV-sterilized water inoculated with VP_{AHPND} to a final concentration of 10^2 to 10^7 cfu/ml. Control shrimp were immersed in sterile seawater, no bacteria were added in the aquaria. The experiments were terminated after 120 h.

Statistical analysis

Significant difference in the cumulative mortality between treatments was determined using repeated measures in SPSS V 23.

Results

Effect of bacterial concentration and exposure method

In the unimmersed group, significantly higher cumulative mortality was observed in shrimp maintained in aquaria with 107 cfu/ml VP_{AHPND} (**Figure 1**). Cumulative mortality in shrimp maintained in tanks with 10^2 to 10^6 cfu/ml VP_{AHPND} and the control were not significantly different. AHPND was detected in shrimp samples from all treatment except the control at 24 and 72 hour post infection (hpi) (**Table1**). At 120 hpi, AHPND was detected only in shrimp maintained at 10^6 and 10^7 cfu/ml. *V. parahaemolyticus* were recovered only in shrimp maintained in water with 10^7 cfu/ml of the bacteria (**Table 1**). VP count

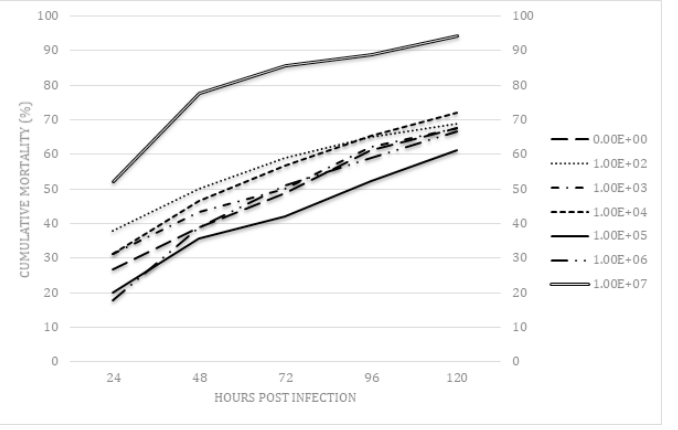

Figure 1. Cumulative mortality in shrimp directly stocked in water with different VP_{AHPND} concentration

in aquaria inoculated to 10^7 cfu/ml was significantly high than the other treatments including the control (**Table 1**). VP count in control aquaria was significantly low compared to treated ones.

In the pre-immersed 2 group, cumulative mortality in shrimp in the negative control was lower, but not significantly, compared to those pre-immersed in VP_{AHPND} (**Figure 2**). VP_{AHPND} was detected in shrimp pre-immersed in bacterial solution at 24, 72 and 120 hpi and maintained in water with 0 to 10^4 cfu/ml VP_{AHPND}, but not in those pre-immersed in sterile seawater (**Table 2**). VP count in the shrimp, including the control were not significantly different. VP count in the water was generally high in aquaria with 10^4 cfu/ml wherein the count increased to 10^5 cfu/ml.

Effect of salinity and temperature

Lower survival was observed in infected and uninfected shrimp maintained at 28 ppt and 10 ppt salinities compared to those at 20 ppt (**Figure 3**). Among the infected group, shrimp survival was significantly low (P>0.05) in those maintained at 28 ppt (54.89 %); highest survival was observed in those maintained at 20 ppt (78.70 %), followed by those at 10 ppt (69.45 %). No significant differences were observed in the mortality of uninfected shrimp maintained at different salinities.

In shrimp maintained at different temperatures, survival was significantly high in those maintained at 31 °C (70.29 %) compared to those at 35 °C (42.07 %) (**Figure 4**). No significant difference was observed in the uninfected group, although, survival was higher in those maintained at 31 °C.

Effect of shrimp age

Using repeated measures analysis (SPSS V.18), observed mortality among the older shrimp bathed in different bacterial concentrations including the control were not significantly different (P>0.05) (**Table 3**). In the younger shrimp, mortalities were significantly high in those bathed in 10^6 and 10^7 cfu/ml. Significantly lower mortalities were observed in the control group (P<0.05) (**Table 3**).

Table 1. Average cumulative shrimp mortality, AHPND detection in shrimp and average VP load of samples in the un-immersed group

Final VP concentration in the water (cfu/ml)	Average Cumulative Shrimp Mortality (%)	AHPND detection in shrimp			Average VP load	
		Hours post infection			Shrimp	Water
		24	72	120	(cfu/shrimp)	(cfu/ml)
10^2	57[a]	+	+	-	0[a]	2.68×10^{1b}
10^3	51[a]	+	+	-	0[a]	5.05×10^{1b}
10^4	54[a]	+	+	-	0[a]	8.54×10^{1b}
10^5	42[a]	+	+	-	0[a]	1.77×10^{1b}
10^6	47[a]	+	+	+	0 [a]	4.25×10^{1b}
10^7	80[b]	+	+	+	2.27×10^{0b}	2.7×10^{3c}
Control	49[a]	-	-	-	0[a]	1.99×10^{0a}

Table 2. Average cumulative shrimp, AHPND detection in shrimp, and average VP load of samples from the pre-immersed group

Final VP concentration in the water (cfu/ml)	Average Cumulative Shrimp Mortality (%)	AHPND detection in shrimp			Average VP load	
		Hours post infection			Shrimp	Water
		24	72	120	(cfu/shrimp)	(cfu/ml)
0	61[a]	+	+	+	2.19×10^2	1.37×10^{1a}
10^2	51[a]	+	+	+	1.48×10^2	1.76×10^{1a}
10^3	65[a]	+	+	+	1.81×10^2	3.21×102^{ab}
10^4	62[a]	+	+	+	1.41×10^2	1.37×10^{5b}
Control	31[a]	-	-	-	3×10^2	1.27×10^{1a}

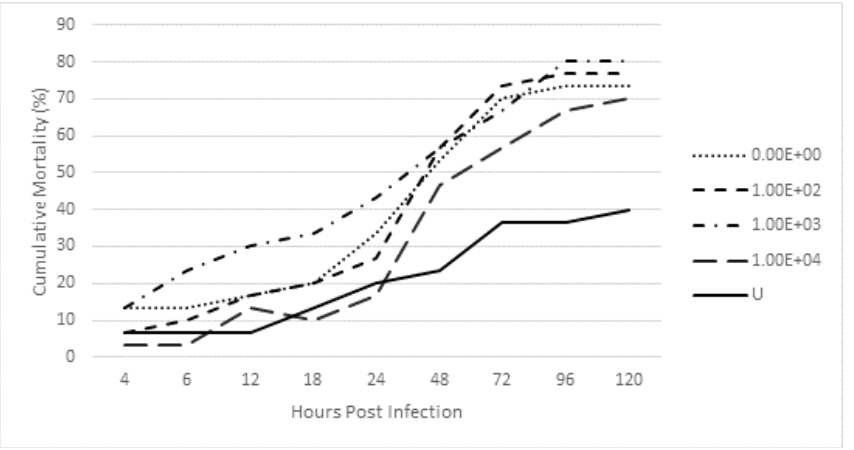

Figure 2. Cumulative mortality in shrimp pre-immersed in VP_{AHPND} prior to stocking in water with different VP_{AHPND} concentration

Discussion

Exposure to 10^7 cfu/ml VP_{AHPND} even for a few minutes is an important risk factor that can result in high mortality in healthy *P. monodon* PL as shown in the results of the infection method studies. The bacteria could have entered the shrimp system, multiply, produce the toxin which increased with the increase in bacterial number and overwhelmed shrimp immune response. This could have led to the irrevocable infection leading to high mortality even if shrimp are cultured in a clean water or water with low bacterial load after exposure. This is validated by the recovery of VP in shrimp cultured in 10^7 cfu/ml VP_{AHPND}, in this study, but not in those cultured in lower concentrations. Results also suggest that VP can multiply inside the shrimp and are excreted into the culture water. Furthermore, shrimp are able to survive VP_{AHPND} infection if exposed to concentrations lower than 10^7 cfu/ml. Results are in consonance with the report that the VP_{AHPND} concentration that the shrimp are exposed to is directly correlated with the mortality rate (Choi *et al*., 2017).

Table 3. Observed cumulative mortality in 2 age groups of *P. monodon* maintained in different concentrations of VP_{AHPND} at 120 hours post infection

Bacterial concentration (cfu/ml)	PL 27 (ABW=0.14)	DOC 150 (ABW=1.3 g)
Control	49[a]	18[a]
10^2	57[a]	35[a]
10^3	51[a]	40[a]
10^4	54[a]	38[a]
10^5	42[a]	29[a]
10^6	47[a]	34[a]
10^7	80[b]	41[a]

The threshold level for VP_{AHPND} bacteria, isolated from the Philippines, in the water that shrimp may overcome is $<10^5$ cfu/ml. This may be attributed to the decrease in the toxin present in the shrimp as implied in the detection of VP_{AHPND} at 24hpc and at 72hpc and not at 120 hpc in those cultured at 10^2-10^5 cfu/ml. The threshold level of the VP_{AHPND} from the Philippines is higher than the reported infectious level of the bacteria isolated from Mexico which is 10^4 cfu/ml (Soto-Rodriguez *et al*., 2015). Furthermore, they noted that different *V. parahaemolyticus* strains have different virulence.

Results confirm anecdotal reports of the effect of salinity and temperature on VP_{AHPND}. The higher mortalities observed in infected and uninfected shrimp maintained at 35°C is consistent with Selven and Philip (2012) who observed higher mortality in *V. harveyi* infected *Fenneropenaeus indicus* maintained at 35 ppt. Higher mortality in infected shrimp maintained at higher temperature confirms that high temperature is a risk factor for AHPND. This may be due to the synergistic effect of high temperature to both the shrimp and the pathogen. As earlier mentioned, higher mortality is observed at higher temperature in penaeids; at the same time, an increase in temperature increases the virulence of the toxic gene present in most bacteria (Guijarro *et al*., 2015). The same mechanism could explain for the effect of salinity on VP_{AHPND} infection in *P. monodon*. *V. parahaemolyticus* toxic genes are better expressed at higher salinity (Alamelu *et al*., 2019) at the same time that shrimp are stressed. The immune ability and disease resistance of *P. monodon* are reduced if transferred to high or low salinity levels (Wang and Chen, 2006). This confirms reports that high salinity is a VP_{AHPND} risk factor.

Mortalities due to VP_{AHPND} seems to be age related as shown in the observe mortalities in the present study. Exposure to as high as 10^7 cfu/ml did not cause mortality in shrimp at DOC 150, but high mortalities were observed in younger shrimp exposed to 10^6 and 10^7 cfu/ml. As shrimp gets older, in the absence of stressor, their susceptibility to VP_{AHPND} decreases as shown in the survival of infected shrimp in the two age groups. Results were in consonance with dela Peña *et al* (2015) who reported 40–60 % mortality in penaeids at DOC 46 and 96, respectively.

To summarize, exposure to 10^7cfu/ml VP_{AHPND}, high temperature (35 °C), and high salinity (28 ppt) increase the risk of shrimp mortality due to VP_{AHPND}. The VP_{AHPND} concentration in the water that *P. monodon* can overcome is $<10^5$ cfu/ml. Mortality due to VP_{AHPND} is age related, higher mortalities observed in younger infected shrimp.

It is recommended that stressors/risk factors that may enhance/stimulate the increase in bacterial population in environments with low bacterial load or toxin production be investigated. Possible preventive measures and treatments should be identified taking into consideration the synergistic effects of the identified stressors/risk factors and bacterial load of the environment.

Acknowledgement

The study was funded by the Government of Japan under the Trust Fund (GoJ TF 6) granted to SEAFDEC/AQD under study code FH04-C2015T. The authors express their gratitude to Ms. Remedios Sobremisana and Mr. Joshua Fabillo for the assistance during conduct of the study.

References

Alamelu V, Sony DMM, Santhosh K, Krishnakumar B, Venugopal MN. 2019. Effect of salinity on the expression of virulent T3SS Gene of *Vibrio parahaemolyticus*. Book of Abstracts: Asian -Pacific Aquaculture 2019. Chennai Tamil Nadu - India.

Bondad-Reantaso MG, Arthur JR. 2018. FAO Technical Assistance Efforts to Deal with Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp. Asian Fisheries Science 31: 01–14

Choi M, Stevens AM, Smith SA, Taylor DP, Kuhn DD.. 2017. Strain and dose infectivity of *Vibrio parahaemolyticus*: the causative agent of early mortality syndrome in shrimp. Aqua Res 4 (7): 3719-3727.

D.V. Lightner, R.M. Redman, C.R. Pantoja, B.L. Noble, L.H. Tran. 2012. Early mortality syndrome affects shrimp in Asia Glob. Aquacult. Advocate, 40.

Daniels NA, Mackiman L, Bishop R, Altekruse S, Ray B, Hammond RM, Thompson S, Wilson, S., Bean, N.H., Griffin, P.M. and Slutsker, L. 2000. *Vibrio parahaemolyticus* Infections in the United States, 1973–1998. The Journal of Infectious Diseases 2000;181:1661–6

Dela Pena LD, Cabillon NAR , Catedral DD , Amar EC , Usero RC , Monotilla WD , Calpe AT, Fernandez DDG , Saloma CP. 2015. Acute hepatopancreatic necrosis disease (AHPND) outbreaks in Penaeus vannamei and *P. monodon* cultured in the Philippines. Diseases in Aquatic Organism 116: 251–254.

FAO. 2013. Report of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304). Hanoi, Viet Nam, on 25–27 June 2013. FAO Fisheries and Aquaculture Report No. 1053. Rome. 54 pp.

Flegel TW. 2012. Historic emergence, impact and current status of shrimp pathogens in Asia. J Invertebr Pathol.110(2):166-73

Flegel TW. 2016. Update June 2016 on AHPND and EHP research in Thailand. FAO Second International Technical Seminar/Workshop on Acute hepatopancreatic necrosis disease (AHPND) There is a way forward! FAO Technical Cooperation Programme: TCP/INT/3501 and TCP/INT/3502 Sukosol HotelBangkok, Thailand23-25 June 2016 pp. 45-46.

Guijarro JA, Cascales D, García-Torrico AI, García-Domínguez M, Méndez J. 2015. Temperature-dependent expression of virulence genes in fish-pathogenic bacteria. Front. Microbiol. 6:700.

Joshi J, Srisala J, Truong VH, Chen I-T, Nuangsaeng B, Suthienkul O, Lo CF, Flegel TW, Sritunyalucksana K, Thitamadee S. 2014. Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). Aquaculture 428-429: 297- 302.

Kondo H, Tinwongger S, Proespraiwong P, Mavichak R, Unajak S, Nozaki R, Hirono I. 2014. Draft genome sequences of six strains of *Vibrio parahaemolyticus* isolated from early mortality syndrome/acute hepatopancreatic necrosis disease shrimp in Thailand, Genome Announc. 2.

Kumar BK, Deekshit VK, Raj JRM, Rai P, Shivanagowda BM, Karunasagar I, Karunasagar I. 2014. Diversity of *Vibrio parahaemolyticus* associated with disease outbreak among cultured *Litopenaeus vannamei* (Pacific white shrimp) in India. Aquaculture 433 (20): 247–251.

Lai HC, Ng TH, Ando M, Lee CT, Chen IT, Chuang JC, Mavichak R, Chang SH, Yeh MD, Chiang YA, Takeyama H, Hamaguchi HO, Lo CF, Aoki T, Wang HC. 2015. Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. Fish Shellfish Immunol. 47(2):1006-14.

Leano EM, Mohan CV. 2012. Early mortality syndrome threatens Asia's shrimp farms. Global. Aquacult. Advocate (2012), pp. 38-39 (July/Aug 2012).

Selven S, Philip R. 2013. Salinity a significant environmental factor for *Vibrio* harveyi virulence in Fenneropenaeus indicus Aquaculture Research 44(5): 747-759.

Tran L, Nunan L, Redman RM, Mohney LL, Pantoja CR, *et al*. .2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis Aquat Organ 105: 45–55.

Wang FI, Chen JC. 2006. Effect of salinity on the immune response of tiger shrimp *Penaeus monodon* and its susceptibility to Photobacterium damselae subsp. damselae. Fish Shellfish Immunol. 20(5):671-81.

Soto-Rodriguez SA, Gomez-Gil B, Lozano-Olvera R, Betancourt-Lozano M, Morales-Covarrubias MS. 2015. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in Northwestern Mexico. Appl Environ Microbiol. 81(5):1689-99.

Summary of Workshop Discussion

The Technical workshop was undertaken to identify research gaps and possible collaboration among ASEAN Member States (AMS). In particular, the participants identified the following:

- Needs and requirements of AMS to promote sustainable aquaculture, aquatic animal health and resource enhancement practices;
- Special needs of AMS for learning procedures and methodologies for the respective practices;
- Application of knowledge and utilization of available resources; and
- Training needs of personnel in member countries

The participants were divided into 2 groups based on their topic of interest or field of specialization: sustainable aquaculture and resource enhancement; and, aquatic animal health (AAH). The participants in both groups answered 4 questions across issues relevant to the topic or group they belong.

The workshop summary for sustainable aquaculture and resource enhancement is presented in **Table 1**.

The AAH group identified 3 key points: disease detection, disease management and systematization. The workshop discussion is summarized in **Table 2**. Research and Development Projects identified during the workshop are the following:

1. Training of professionals for disease surveillance and management.
    - 1.1. Training: In-country/On-site Training to enhance capabilities
    - 1.2. Disease Surveillance of Emerging and Endemic Diseases
        - 1.2.1. Shrimp: EHP, WFD, AHPND, WSSV and SHIV
        - 1.2.2. Freshwater Fish: Tilapia (TiLV) and Streptoccocal Infection
        - 1.2.3. Marine Fish: Fish Iridovirus and Vibriosis

2. Training of professionals for proper use of antimicrobials for aquaculture applications and monitoring/preventing emergence of antimicrobial resistance.

3. R&D efforts in developing platform technologies in delivering therapeutics via oral route against major diseases (e.g. WSSV in shrimp, TiLV for Tilapia, and Streptococcal Infection).

4. Development of point-of-care diagnostics.

The AAH group recommended the enforcement of established guidelines related to aquatic diseases (e.g. on disease surveillance and disease reporting, etc.), adaption of established guidelines for food safety and traceability, and enhancement of collaboration and sharing of information and knowledge among AMS.

Table 1. Workshop Summary – Sustainable Aquaculture and Resource Enhancement

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
Low fish production	• Occurrence of fish diseases	• Lack of technical manpower to do aquatic animal disease surveillance	• Recruitment of trained personnel on fish health management	• Capacity building of concerned personnel on the diagnosis, prevention and control of emerging diseases in fish, shrimps and crustaceans
		• Inadequate government facilities for disease surveillance in SEAFDEC member countries (e.g. Malaysia, Lao PDR, Cambodia, Thailand, Myanmar, Philippines, Indonesia)	• Promotion of public-private partnership to strengthen laboratory diagnostic services	• Support cost-effective services in strategically located public and private laboratories • Develop reliable, low-cost testing kits for disease monitoring
		• Insufficient biosecurity measures practiced in aquaculture farms	• Increased understanding and satisfactory implementation of biosecurity measures in aquaculture farms	• Proper planning and mandatory implementation of biosecurity measures in aquaculture farms
	• Low, poor quality and inconsistent seed supply	• Lack of good quality grouper and snapper (Myanmar and Indonesia); prawn and shrimp (Myanmar); white shrimp (Thailand); milkfish, tilapia, shrimp (Philippines); grouper, snapper, and pompano (Singapore) broodstock for use in seed production	• Compliance to proper farm management practices • Development of seed production techniques for dissemination to fish farmers • Development of broodstock with improved reproductive traits	• Encourage government to provide continued support to fish farmers to improve fish production by producing quality seeds

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
		• Lack of trained hatchery personnel in broodstock management	• Importation of better quality broodstock from other countries with good quarantine system in place as a short-term solution (e.g. Myanmar)	• Conduct of training on broodstock management with technical support from other SEAFDEC member countries
		• Unsustained fund support and uncoordinated investments for research and development projects on domestication and selective breeding in fish	• Application of genetic modification techniques in selective breeding of fish • Improvement of research capability through upgrading of facilities and equipment and training on genetic manipulation tools	• Upgrading of laboratory/ training facilities
		• Lack of cost-effective diets for broodstock, larval and grow-out stages	• Development of alternative feeds for various developmental stages of fish using locally available feed ingredients	• Implement research programs involving SEAFDEC member countries on the development and use of alternative protein sources in diets for aquaculture species
		• Low or poor government support for the sustained operation of hatcheries producing even at low capacity	• Promotion of mobile hatchery as initiated in Lao PDR	• Enhance capacity of hatcheries through hiring of additional production staff • Improve and ensure sustainability of government hatchery facilities • Promote private hatcheries to augment production of seedstock
		• Weak implementation of quarantine protocols and inadequate containment facilities for imported shrimp postlarvae (Myanmar and Cambodia) and broodstock (Philippines)	• Implementation of programs for monitoring diseases in shrimp postlarvae and broodstock	• Apply biosecurity measures to prevent the entry of pathogens in fish farm facilities

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
	• Declining wild fishery catch	• Weak implementation of fishing regulation (e.g. Myanmar) • Insufficient government budget (all SEAFDEC member countries) • Weak prioritization of fisheries resource protection (Japan and all SEAFDEC member countries except Singapore)	• Increase number of trained personnel to be involved in fisheries resource management • Enhance awareness of fisheries resource protection through inter-agency collaboration	• Convince the governments to prioritize the fisheries sector
	•Dependency on wild-sourced stocks (e.g. juveniles) for culture production	• Low survival for marine fish in the hatchery phase • No data collection for fishery stock assessment	• Needs studies on stock assessment to enable the proper management of wild stocks • Conduct research on freshwater fisheries resources (e.g. Myanmar) and proper recording and updating of data for research use	• Implement research programs on stock/ resource enhancement in areas where there have been reported decline in natural populations
		• High cost of production due to inappropriate husbandry practices (e.g. high stocking density)	• Development of new technology to support the management strategy	• Develop technology or programs for use in management of aquaculture operations

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
	Non-compliance to Good Aquaculture Practices (GAqP)	• Lack of strict implementation of GAqP in aquaculture farms in SEAFDEC member countries (e.g. Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore) for there are no penalties imposed for non-compliance	• Conduct of training on GAqP for fish farmers to increase awareness and improve adoption of good management practices	• Learn from success stories and adopt the best aquaculture practices from Thailand
		• Cumbersome process and high costs for GAqP certification	• Streamlining of GAqP certification process (e.g. Philippines) • Government to subsidize the high cost of GAqP certification to support the fish farmers for the certification	• Incentivize certification by selling a product at a premium price to help farmers increase their profits • Encourage establishments and/or consumers to buy fishery products from fish farms with GAqP certification • Create market segmentation to improve profitability and enhance fishery product competitiveness in the market
		• Lack of trained personnel to assess the implementation of GAqP in aquaculture farms in respective SEAFDEC member countries	• Recruitment and training of additional personnel to inspect aquaculture farms and ensure that the best management practices are effectively implemented	

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
	• Environmental degradation/ eutrophication/ Harmful algal bloom (HAB)/ Climate variation	• Irresponsible human activities • Lack of human capacity experience • No proper zoning plan or site assessment to identify areas suitable for aquaculture	• Training on environmental monitoring and responsible aquaculture practices • Studies on the impacts of IMTA, RAS, offshore aquaculture, and biofloc system needed to achieve environment-friendly and sustainable production systems • Application of environmental modelling and GIS-based tools for monitoring • Development of contingency plans for HABs with funding from government agencies	• Need collaboration among SEAFDEC member countries on studies of environmental conditions • Transformation of aquaculture industry into a high-tech sustainable industry
		• Lack of carrying capacity assessment/ data for fish production regions	• Need to conduct site selection and carrying capacity studies in areas intended for aquaculture	
		• No regular environmental monitoring program for existing fish farms		
		• Poor farm management practices (feeding/ choice of feeds)	• Elucidation on the impact of feed types (floating vs sinking) on water quality • Determination of optimal feeding rate for rearing of various fish species	
		• Resource-use conflict vs agriculture industries, factories, etc)	• Better inter-agency coordination to properly address resource-use conflicts	

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
Food safety and traceability of fish and fishery products	• Low compliance to food safety and quality standards • Malaysia – standards are in place for export products are for such as shrimp, ornamental fish, and marine fish •Thailand, Philippines and Singapore – developed standards mainly for export products • Myanmar – adopted EU guidelines • Lao PDR (on experimental scale) and Cambodia – no standards in place	• Compliance to food safety requirements not mandatory for local consumption	• Promote eco-labeling in products intended for export and even for local consumption	• Incentivize compliance to food safety and quality standards (e.g. HACCP) • Promote consumers' awareness and education about the importance of eco-labeling

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
Legal Framework/ Policy and regulation	• Resource use conflicts in areas with aquaculture activities	• Issues related to land and water use fall under the jurisdiction of state especially local authority	• Promote the adoption of fisheries in inland water bodies (e.g. Malaysia) to control aquaculture activities efficiently and effectively	
		• Weak/ low enforcement to address resource use conflicts	• Establish a separate ministry (e.g. Malaysia) to ensure effective implementation of laws to address conflicts between various stakeholder groups	• Cooperate with the ministry for effective execution of laws pertinent to resource related conflicts
	• Uncontrolled aquaculture activities that leads environmental issues such as pollution	• Lack of base map and decision tools (e.g. marine spatial planning) for regulating the impacts of aquaculture activities on the environment	• Implementation of Ecosystem Approach to Fisheries Management (EAFM) in thecontext of Aquaculture and Resource Enhancement • Improve awareness of fisheries communities to EAFM	• Strengthen the EAFM/ research in collaboration with national and international agencies • Strict law enforcement
			• Use of closed system aquaculture in producing various aquatic commodities	• Dissemination of closed system aquaculture technology for possible adoption of fish farmers
Overfishing and depletion of valuable stocks	• Scarcity of land areas for farming or for rotation farming as alternate non-fishing livelihood	• Unlimited resources: land area • Compare farming, fishing and aquaculture with other industries that have more opportunities to generate income	• Convince local authorities such as economic planning unit to allocate more budget and areas for aquaculture	• Give sufficient inputs such as applicable technology based on projection of positive economic result

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
	• Less development of marine aquaculture due to limited human resource and capacity	• Low salaries for hatchery personnel	• Provision of high salaries and benefits to motivate aquaculture staff	• Development of personnel in the fisheries sector through training and extension work
		• Inadequate trained personnel	• Provide opportunities for training, education and extension work	
		• Inadequate facilities in marine hatcheries	• Upgrade in research and production facilities	• Collaboration in hatchery research and training, and provision of support services and facilities
	• Lack of funds and technical capabilities of LGUs and fisherfolk communities to sustain post-project implementation of RE initiatives	• Political will, particularly during times of transition, is unpredictable	• Apply value chain analysis as a tool to understand the business side or RE and overall promotion of RE initiatives among stakeholders	• Conduct value chain analysis to promote livelihood creation out of RE initiatives among constituents
		• Business side of resource enhancement not fully articulated in spite of benefits from often high-value aquatic products	• Engage Corporate Social Responsibility (CRS) or Private Foundations as the "Third force" in RE initiatives • Engage local educational institutions as a force multiplier on technical aspects of RE initiatives	• Consultations with corporations to promote RE partnerships (conservation, livelihoods, scientific goals) • Capacitate, incentivize local colleges/ universities to take-on (continue) scientific work of RE
	•Lobster fishery is under threat of overfishing	• Lobster fisheries and current aquaculture practices not fully understood	• Development of science-based lobster fishery management, at least in key local sites in the Philippines (e.g. CARAGA/ Surigao Provinces) and other member countries	• Population studies (biomass, breeding population, larvae recruitment) on local lobsters and habitat protection

Keypoints and Issues	What is the problem?	Why has the problem not solved yet?	What is possible?	What shall we do next?
			• Promote sustainable lobster aquaculture practices in SE Asian countries with wild lobster breeding population (e.g. Philippines, Viet Nam, Indonesia)	
	• Lobster feeding, and over-all farming system needs improvement	• Limited information available on the biology and rearing techniques for lobster, although local sites in the Philippines were already identified	• Promote sustainable lobster- based livelihoods in key localities	•Development of supplemental or formulated feed for lobster culture •Conduct of value chain analysis of local lobster resources
	•Over-exploitation of mangrove crabs apparent (e.g. Catanduanes Province, Philippines)	• Implementation of the Philippine national law RA 10857 that will enable Catanduanes and similar sites to improve its mangrove crab fisheries • Value chain analysis needs review	• Technology transfer activities (e.g. demonstration, on-site training, study tour) • Investments to improve existing brackishwater ponds • Development of farm-made feeds to sustain mangrove crab culture • Development of science-based mangrove crab fishery management	• Population studies (biomass, breeding populations, larvae recruitment) • Review value chain analysis in view of creating livelihoods out of RE initiatives • Consultations with corporations to promote RE partnerships (conservation, livelihoods, scientific goals) • Capacitate and incentivize local educational institutions to pursue scientific work on RE and aquaculture extension

Table 2. Workshop Summary - Aquatic Animal Health

Key points and Issues	What is the problem	Why has the problem not been solved yet	What is possible?	What shall we do next?
Disease detection	• Disease detection capability • Detection of exotic and emerging disease in a timely manner	• Lack of technical expertise for identifying emerging diseases	• Further enhance capacity building (e.g. strengthening of NGO's) • Enhance collaboration and training of technical experts	• Network with neighboring countries • Seek support from technical experts from within and outside the country (ASEAN mechanism)
			• Enhance Proficiency testing and harmonize disease detection programs among ASEAN member states	• Increase collaboration with other countries (ASEAN member states) having good technical expertise • Seek support to initiate Twinning Projects through OIE
	• Unavailability of affordable/user friendly point- of-care diagnostics	• Lack of enough R&D efforts to develop point-of-care diagnostics and therapeutics	• Consider emerging needs on the ground for point-of-care diagnostics and therapeutics • Research can be custom-tailored to address farmers' needs	• Support point-of-care diagnostics • Focus on developing therapeutics • Seek support from local and national agencies for the training /capacity building of staff
	•not enough trained personnel	• Inadequate resources to training and capacity building • Frequent re-assignment of personnel	• Increase the number of trained personnel and technical expertise • Retain trained personnel to assigned tasks for which they were trained • Assign responsibility to trained personnel and target accomplishing task on time	• Conduct on-site training

Key points and Issues	What is the problem	Why has the problem not been solved yet	What is possible?	What shall we do next?
Disease management	• Selection of appropriate site for aquaculture farms	• Lack of alignment of priorities based on needs i.e, site assessment prior to pond construction	• Environmental impact and Hydrodynamic studies before initiating aquaculture operations	• Information, Education, Communication campaign on GAP which includes site selection
	• Inadequate implementation of biosecurity protocols	• Lack of knowledge in biosecurity	• Enhancement of extension services • Lack of Initial screening of seeds for aquaculture farming • High quality seeds not available (disease-free seeds) • Limited access to culture technologies and disease management strategies	• Conduct on-site training • Improvement of production protocols • More active extension services to the farmers • Specialized training for Veterinary Professionals in Aquaculture practices and techniques
	• Information gap on risk assessment related to spreading of disease • Unwillingness of farmers to report disease outbreak • Inadequate active disease surveillance	• Knowledge gap on the effect of the irresponsible water waste disposal • No coordinated effort for epidemiology program	• Encourage cluster-based farming approach • share information within the cluster • Conduct cohort and case control studies to determine risk factors on the spread of diseases • Increase active disease surveillance and reporting • Enhance networking among laboratories with other Asian countries to enhance capacity building	• Train farmers with in the cluster on sustainable and responsible farming • Enhance disease surveillance and timely reporting of disease outbreak

Key points and Issues	What is the problem	Why has the problem not been solved yet	What is possible?	What shall we do next?
	• Inadequate R&D on fishmeal-based replacement diets	• Finding cost-effective alternative to fishmeal diet without compromising animal growth	• Enhance the long-term funding for applied research in developing therapeutics for farm level applications	• Enhance nutritional quality of aquaculture diets • Enhance utilization of regional database on alternative ingredients for aqua feeds
Systematization	• Insufficient long-term programs to support capacity building • Information sharing among stakeholders and among other countries • Inadequate implementation of guidelines for disease detection and management response	• Inadequate financial support • Very limited research support	• Strengthening the concerned national agencies • Enhance networking among laboratories from other Asian countries	• Seek additional support to further enhance AAH from national agencies • Information sharing • Advocate implementation of ASEAN guidelines for disease management • Follow relevant ASEAN guidelines on responsible movement of live aquatic organisms

International Workshop on Fish Health and Sustainable Aquaculture

"Promotion of sustainable aquaculture, aquatic animal health, and resource enhancement in Southeast Asia"

25-27 June 2019

Richmonde Hotel, Iloilo City, Philippines

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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 Terengganu, 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 Fresh water Station in Rizal province. AQD also has an office in Quezon City.


Tigbauan Main Station


Igang Marine Station


Dumangas Brackishwater Station


Binangonan Freshwater Station

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