ADVANCES IN AQUACULTURE

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Centre of Research for Advanced Aquaculture (CORAA)

Edited by: Jiun-Yan Loh, Michelle Oi-Yoon Soo, Baskaran Gunasekaran and Victor Charlie Andin

UCSI University

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Preface

Global aquaculture has grown dramatically over the past 50 years and the trend is continually suppressing other animal production industry, making it the fastest-growing food production sector in the world. With huge seafood demands for a steadily growing population in 2050, the advancement of aquaculture production methods is vital. Modern farming practices and biotechnology are constantly being applied in the aquaculture sector to address various production limitations such as the supply of juveniles from hatcheries, nutritional quality, and etc.

This book "Advances in Aquaculture" provides a comprehensive, systematic guide to the use of current and emerging technologies in aquaculture production. The authors reviewed current technologies in algal production as a primary food source for fish and crustacean larvae. With the advancement of aquaculture technology, invertebrate production can be limitless in terms of stock quality and productivity. Nutrition is one of the major constraints in current aquaculture; some important nutritional issues emphasis in finfish and shellfish were discussed, the baseline information is of particular importance to the farmers. Last but not least, disease management is another biosecurity measure to ensure continuity and sustainability of aquaculture. With a range of upcoming and distinguished editors and expert contributors, "Advances in Aquaculture" is an authoritative review of the field for modern aquaculture.

This book would not have been possible without the dedicated efforts of the chapter authors. We hope this book will be able to stimulate research and better understanding of technological advances in growth, reproduction, and fish health management as more and more evidences show that aquaculture is the best animal production industry for protein and food to cater to the demand of a growing population in the world.

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Chapter 1

ADVANCES IN SEAWEED CULTIVATION WITH FOCUS ON THE TROPICS

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ABSTRACT

Over the last 50 years, the rapid development of seaweed cultivation technologies in tropical countries has resulted in the seaweed aquaculture industry producing more than 99% of seaweed biomass production in the tropics. In 2020, the major farmed seaweed species in the tropics, namely *Eucheuma* spp. (8,194,053.60 tonnes FW), *Gracilaria* spp. (5,176,744.42 tonnes FW), and *Kappaphycus alvarezii* (1,603,213.33 tonnes FW), accounted for 98.92%, 99.90%, and 98.92% of the respective species' global aquaculture production, with USD 5,653,007.86 in value. The main methods of growing seaweed were line cultivation, floating raft cultivation for *Eucheuma* spp. and *Kappaphycus* spp., and open water and pond cultivation for *Gracilaria* spp. These methods have been used in selected tropical countries since the 1970s and 1980s. However, there are still many improvements needed and countless challenges to overcome for the sustainable integration of seaweed cultivation production into the new era of the global aquaculture industry.

Keywords: Seaweed Cultivation, Algae Biotechnology, Seaweed Aquaculture, *Eucheuma, Gracilaria, Kappaphycus alvarezii*

1.1 Introduction

Seaweeds are benthic macroalgae present in marine habitats. Seaweeds are found along the shores of all areas, from the tropics to the polar regions, and play an important part in the ecology of the ocean. The macroalgae are thallophytes, which implies that they do not have well-defined vascular tissues, whilst lacking roots, stems, and leaf structures. Nevertheless, they do have chlorophyll as a major photosynthetic pigment. Seaweeds also do not possess complicated reproductive systems and do not have sterile cells in their reproductive systems, meaning that all cells are potentially fertile (Lee, 1999; Barsanti & Gualtieri, 2006, Baweja et. al. 2016). Seaweeds serve as primary producers in the food chain of the marine ecosystem. As such, they are an essential source of oxygen, carbon dioxide, and water for the metabolic processes of marine organisms. They produce organic materials from sunlight too (Lee, 1999; Baweja et. al. 2016). Seaweeds can be classified into three main groups, namely green seaweed (Chlorophyta), brown seaweed (Ochrophyta) and red seaweed (Rhodophyta) based on their characteristic pigmentation. There are approximately 1,800 species of green seaweed, 6,200 species of red seaweed, and 1,800 species of brown seaweed reported found in the natural environment (Mouritsen, 2013).

Today, seaweed is one of the marine resources that has received substantial interest, particularly in developing countries. This is due to the high demand for seaweed as a source of food for direct human consumption, animal feed in the agriculture and aquaculture industries, fertilizer and soil conditioner in the agriculture business, and a source of industrial components in the food and medical industries. Furthermore, because of the intensive development of algae biotechnology, seaweed has emerged as a source of bioactive natural compounds that may have value in the nutraceutical, cosmeceutical, and pharmaceutical industries. Seaweed is also a source of biomass that can be used in the production of alternative bioenergy and biofuels.

The increasing demand for seaweed resources as raw materials from various industries globally has led to an increase in world seaweed production and utilization. The annual global production of seaweed increased from 2,116,809 tonnes (fresh weight) in 1950 to 36,165,326 tonnes (fresh weight) in 2020 (FAO, 2022b), representing an increase of around 8879.87% over the course of the same period. This biomass of seaweed was collected either from natural populations or from the seaweed aquaculture industry. The rapidly increasing demand for seaweed biomass has also resulted in the expansion of the seaweed aquaculture business and the development of techniques for seaweed cultivation, both of which are intended to increase the amount of seaweed produced through seaweed aquaculture. This led to a rise in the number of interested parties and investors who invested in the development of advanced cultivation technologies for the seaweed aquaculture industry (García-Poza et. al. 2020).

1.2 Seaweed Production

The annual world seaweed production can be attributed to the harvesting of seaweed from both wild populations and farming industries. In 1950, the annual world seaweed production amounted to 407,273 tonnes fresh weight (FW), of which 91.50% (372,637 tonnes FW) was taken from the wild populations and 8.50% (34,636 tonnes FW) was harvested by the seaweed aquaculture industry. The annual production of seaweed had climbed to 2,116,809 tonnes FW by the year 1970. Of this total, 54.06% came from farmed seaweed (1,144,380 tonnes FW), while 45.94% was collected from the wild population (972,429 tonnes FW). Since 1970, there has been a substantial increase in the annual world seaweed production (Figure 1.1).

In 2020, world seaweed production achieved an annual output of 36,165,326 tonnes FW, of which 96.81% (35,013,088 tonnes FW) was produced by the seaweed aquaculture farming industry, and this aquaculture production was worth an annual value of USD 16,444,926,480. In 2020, the highest seaweed aquaculture production was *Laminaria japonica* (12,469,807 tonnes FW), and followed by *Eucheuma* spp (8,283,513 tonnes FW), *Gracilaria* spp (5,180,416 tonnes FW), *Undaria pinnatifida* (2,810,605 tonnes FW), *Porphyra* spp (2,220,180 tonnes FW) and *Kappaphycus alvarezii* (1,604,110 tonnes FW) (FAO, 2022b).

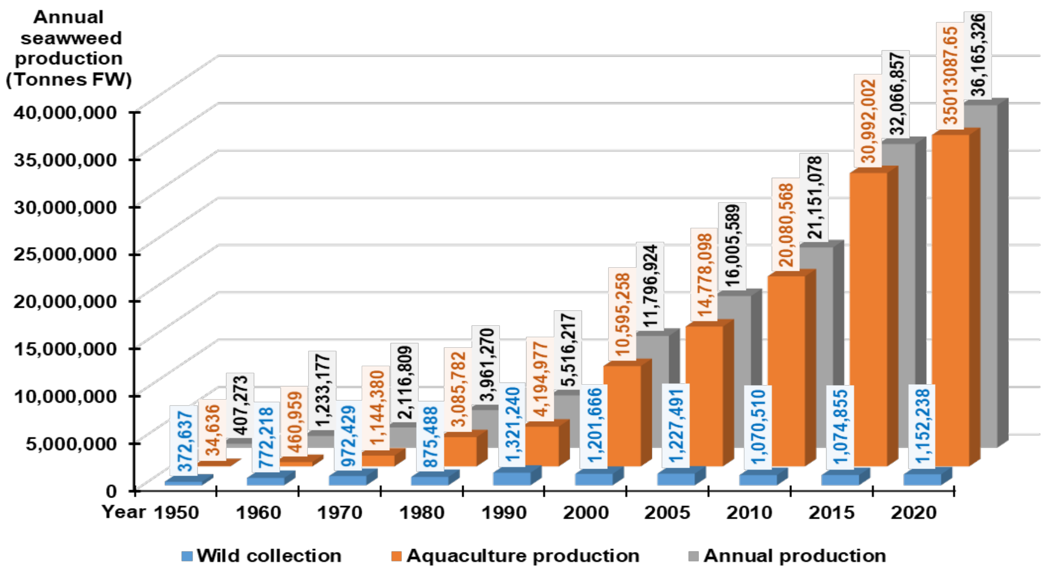

Figure 1.1 World seaweed production (1950 -2020) (Data extracted from FAO, 2022).

In the tropical region, the annual seaweed production started to increase greatly after the 1970s, when seaweed farming was introduced to the tropics. In 1950, the annual production of tropical seaweed was 17,060 tonnes FW, of which 94.31% (16,090 tonnes FW) was harvested from wild populations and 5.69% (970 tonnes FW) came from seaweed farms (Figure 1.2). In 1980, seaweed aquaculture production started to dominate the seaweed production in the tropics, contributing to 61.83% (143,150 tonnes FW) of the annual seaweed production in the tropics. Since 2005, tropical seaweed aquaculture has contributed more than 95% of the annual seaweed production in the tropics. In 2020, 99.08 % (15,061,383 tonnes FW) of total annual seaweed production in the tropics was harvested by tropical seaweed aquaculture farming, with USD 5,681,861.48 in value (FAO, 2022a).

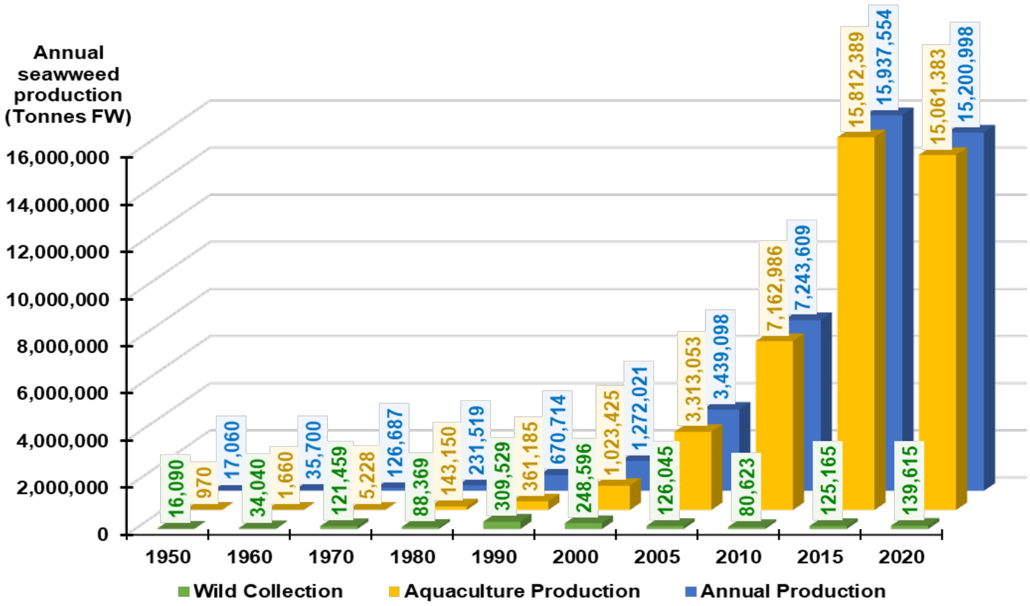

Figure 1.2 Tropical seaweed production (1950 -2020) (Data extracted from FAO, 2022).

In spite of the fact that tropical nations produced approximately 35.92% of worldwide seaweed aquaculture production in 2020, only three major seaweeds are actively cultivated in tropical regions. The annual tropical seaweed aquaculture production from 1985 to 2020 is summarized in Table 1.1. In 2020, the major farmed seaweed species in the tropics, namely *Eucheuma* spp. (8,194,053.60 tonnes FW), *Gracilaria* spp. (5,176,744.42 tonnes FW), and *Kappaphycus alvarezii* (1,603,213.33 tonnes FW), accounted for 98.92%, 99.90%, and 98.92% of the respective species' global aquaculture production. Table 1.2 summarizes the major seaweed production (tonnes (FW) and value (USD)) and the top three producing countries in the tropics.

Table 1.1 Annual seaweed production (tonnes FW) and value (USD) in the Tropics (1985 - 2020)*.

No	Seaweed	1985	1990	1995	2000	2005	2010	2015	2020
1	***Caulerpa* spp (tonnes FW)**	**11,278.00**	**17,471.00**	**4,040.00**	**28,296.00**	**4,309.00**	**4,309.00**	**1,219.00**	**1,021.36**
	***Caulerpa* spp (USD)**	**3,637.22**	**4,699.63**	**458.62**	**3,367.62**	**1,549.38**	**2,560.97**	**1,073.99**	**623.34**
2	***Eucheuma* spp (tonnes FW)**	**9,668.00**	**26,939.00**	**19,245.00**	**249,730.00**	**1,089,116.00**	**3,611,634.40**	**10,295,622.60**	**8,194,053.60**
	***Eucheuma* spp (USD)**	**1,211.49**	**3,183.11**	**3,263.51**	**25,449.03**	**166,896.05**	**1,147,173.44**	**806,508.91**	**1,684,999.44**
3	***Gracilaria* spp (tonnes FW)**	**6,524.00**	**40,057.00**	**57,222.00**	**51,514.00**	**933,184.00**	**1,657,144.00**	**3,767,020.00**	**5,176,744.42**
	***Gracilaria* spp (USD)**	**1,999.60**	**17,500.62**	**22,184.65**	**17,692.41**	**276,706.87**	**589,445.65**	**1,614,045.11**	**2,359,412.56**
4	***Kappaphycus alvarezii* (tonnes FW)**	**162,385.00**	**259,247.00**	**562,043.00**	**649,464.00**	**1,279,234.80**	**1,884,024.10**	**1,746,008.30**	**1,603,213.33**
	***Kappaphycus alvarezii* (USD)**	**28,236.23**	**37,373.63**	**53,452.63**	**47,828.15**	**107,167.66**	**266,368.59**	**212,654.87**	**1,608,595.86**
5	**Others (tonnes FW)**	**0.00**	**0.00**	**0.00**	**0.00**	**16,125.00**	**2,900.00**	**1,565.00**	**1,300.00**
	Others (USD)	**1,843.22**	**5,000.00**	**11,220.00**	**1,824.69**	**1,234.09**	**641.29**	**462.29**	**28,230.28**
	Total (tonnes FW)	**135,186.00**	**189,855.00**	**343,714.00**	**642,550.00**	**995,129.00**	**3,308,743.80**	**7,158,676.50**	**15,811,169.90**
	Total (USD)	**36,927.76**	**67,756.99**	**90,579.41**	**96,161.90**	**553,554.05**	**2,006,189.94**	**2,634,745.17**	**5,681,861.48**

*Data extracted from FAO. 2022. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2020 (FishStatJ). In: FAO Fisheries and Aquaculture Division [online]. Rome. Updated 2022.

Table 1.2 Major seaweed production (tonnes FW) and value (USD) in the Tropics (2000 and 2020)*.

No	Country*	2000		2020	
		Production (tonnes FW)	Value (USD)	Production (tonnes FW)	Value (USD)
1	***Eucheuma* spp**				
	Indonesia	**197,227.00**	**21,694.97**	**8,080,796.00**	**1,640,850.95**
	Philippines	**34,424.00**	**2,953.24**	**63,073.20**	**8,066.93**
	China	**0.00**		**38,560.00**	**32,351.84**
	Total	**249,730.00**	**25,449.03**	**8,194,053.60**	**1,684,999.44**
2	***Gracilaria* spp**				
	China	**0.00**	**0.00**	**3,689,670.00**	**2,025,628.83**
	Indonesia	**8,000.00**	**880.00**	**1,456,730.00**	**117,279.94**
	Chile	**33,471.00**	**16,735.50**	**18,268.00**	**216,475.80**
	Total	**51,514.00**	**17,692.41**	**5,176,744.42**	**2,359,412.56**
3	***Kappaphycus alvarezii***				
	Philippines	**644,319.00**	**46,962.78**	**1,404,743.36**	**205,300.02**
	Malaysia	**0.00**	**0.00**	**182,061.00**	**1,400,419.70**
	Solomon Islands	**0.00**	**0.00**	**5,500.00**	**205.58**
	Total	**649,464.00**	**47,828.15**	**1,603,213.33**	**1,608,595.86**
4	***Caulerpa* spp**				
	Philippines	**28,296.00**	**3,367.62**	**789.36**	**515.38**
	Indonesia	**0.00**	**0.00**	**232.00**	**107.96**
	Total	**28,296.00**	**3,367.62**	**1,021.36**	**623.34**

* The top 3 seaweed producing countries in the tropics in 2020

**Data extracted from FAO. 2022. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2020 (FishStatJ). In: FAO Fisheries and Aquaculture Division [online]. Rome. Updated 2022.

1.3 Seaweed Utilization

Since 2500 BC, seaweed has been utilized in China (Tseng, 1981). However, Hoppe and Schmid (1969) state that the first few true instances of economic exploitation of seaweed were carried out in China and Japan around the year 1670. There have been at least 291 different types of seaweed utilized on a global scale up to this point. This includes 33 species of green seaweed, 75 species of brown seaweed, and 163 species of red seaweed, as reported by Lindsey White and Peter Wilson (2015) (Table 1.3). In 2019, the world seaweed aquaculture industry was cultivating a total of 27 different seaweed species (items as from ASFIS8 (database of the Aquatic Sciences and Fisheries Information System), but only 3 major seaweed groups or 5 seaweed species were cultivated in tropical regions (Cai et al, 2021), including *Eucheuma* spp., *Gracilaria* spp. and *Kappaphycus alvarezii.*

Table 1.3 Summary of the composition, important properties, uses, and values of the commercially important phycocolloids seaweeds (*Eucheuma spp, Gracilaria spp., and Kappaphycus alvarezii)**.

Seaweeds	Uses and Applications
Eucheuma spp, ***Kappaphycus* spp.**	**Carrageenans** • *Kappaphycus alvarezii* - important source of kappa carrageenan • *Eucheuma denticulatum* - important source of iota carrageenan • **Composition** ▪ Kappa- and iota- ▪ Alternating 1,3-linked α-D-galactosa and 1,4-linked 3,6-anhydro-β-D-galactosa backbone (carrabiose) substituted with varying percentages of ester sulphate. • **Important properties** ▪ Bind moisture. ▪ Stabilize emulsions ▪ Control flow and texture properties of food systems. ▪ High protein reactivity- strong interactions with milk proteins • **Selected application** ▪ dairy products: kappa acarrageenan added in cottage cheese/ ice cream to prevent separation & control texture ▪ Water-based foods: as substitutes for gelatin; suitable for vegetarians ▪ meat products: carrageenan binds water within the poultry muscle and improves texture and tenderness. ▪ Frozen dessert stabilizers. ▪ Chocolate milk stabilizer. ▪ Texturizers for low fat foods. ▪ Low-calories jellies. ▪ Toothpaste binders. ▪ Air-freshener gels. ▪ Personal care products. ▪ Immobilized biocatalysts.
***Gracilaria* spp.**	**Agar** • **Composition** ▪ Alternating 1, 4-linked α-D galactose and 3,6 anhydro-α-1 galactose backbone (agarobiose) substituted with varying percentages of methoxyl, ester sulphate and ketal pyruvate groups • **Important properties** ▪ Gel aqueous at low concentrations. ▪ Form thermo reversible gels. ▪ Relatively inert. ▪ Significant degree to hysteresis. ▪ Retain moisture. ▪ Resist hydrolysis by terrestrial microorganisms. ▪ Belong to galatans. ▪ Give viscosity to aqueous solutions.

	• **Selected application** ▪ Dental impression media ▪ Jelly candies ▪ Baking icings ▪ Jams and soups ▪ Canned meat ▪ Laxatives ▪ Microbial culture media ▪ Raw material for agarose ▪ Textile sizing and block printing **Agarose** • **Composition** ▪ Alternating 1, 4-linked α-D galactose and 3,6 anhydro-α-1 galactose backbone (agarobiose) substituted with varying percentages of methoxyl, ester sulphate and ketal pyruvate groups • **Important properties** ▪ Gel aqueous at low concentrations. ▪ From ion-dependent thermo reversible gels. ▪ Controllable electroendosinosis. ▪ Minimal non-specific protein reactivity. ▪ Significant degree of hysteresis. • **Selected application** ▪ Material for: Electrophoresis, Immunoassays ▪ Microbial and cell culture ▪ Chromatography ▪ Immobilized system

*Modified from Jensen, 1993; Renn, 1997; McHugh, 2002; Phang et al 1996; Phang et al 2010a & 2010b; Bixler & Porse, 2010 and Phang, 2010)

Aside from that, seaweed has a greater potential for the production of bioplastics than bacterial and plant sources because it is extremely rich in polysaccharides such as carrageenan, agar, floridean, starch, and alginate. This is because seaweed is able to convert its starch into alginate. These chemicals provide adequate properties to make edible bioplastic films and packages with high tensile strength, flexibility, and elasticity. These qualities are desirable in bioplastics (Moey et al., 2015). The usage of kappa-carrageenan, which is derived from Rhodophyta seaweed, has been introduced as a precursor for the development of bioplastic film. This can improve the moisture barrier and tensile strength of conventional bioplastic films (Abdul Khalil et al., 2017). Conventional plastics that land up in landfills are the root cause of today's plastic epidemic and may be completely broken down into its component parts by bioplastics made from seaweed at a far faster rate. This may provide a sustainable solution to the problem that conventional plastic presents.

Due to the high concentration of rich polysaccharides and the gelling properties of seaweed, it is frequently used as the primary ingredient or as an additive in a variety of industries and applications, including the food industry (Figure 1.3), industrial applications, agriculture, skincare, and therapeutic fields (Ganesan et al., 2019; Cherry et al., 2019). Recent developments in water bioremediation have seen an increased emphasis placed on the utilization of natural materials or biomass. In order to find a suitable replacement for chemically-manufactured adsorbents, seaweed has been proposed as a possible biosorbent for the removal of pollutants in water bioremediation. These pollutants include textile dyes, heavy metals, and organic wastes (Oualid et al., 2020; D. Shah et al., 2021; Buhani et al., 2021). According to a study that was conducted by Abdullah Al-Dhabi and Arasu (2022), the phytoremediation process used by marine seaweed was able to effectively remove heavy metals from wastewater such as lead (Nessim et al., 2011; Eka Putri et al., 2018; Isam et al., 2019).

In recent research, seaweed has been utilized for surface modification in magnetic nanoparticle adsorbents in order to improve the adsorbent's stability. This was accomplished by increasing the surface area of the adsorbent. Research into the adsorption of proteins and the removal of dyes using magnetic nanoparticles with kappa-carrageenan as a surface modifier has been conducted (Mahdavinia & Etemadi, 2019; Salgueiro et al., 2013; Soares et al., 2017). The coating layer not only helps to improve the colloidal stability of the nanoparticles but also works as a binder to adsorb heavy metals. This is one of the many roles that the coating plays (Wan et al., 2021). Electrostatic attraction was one of the potential mechanisms that may be used to accomplish the goal of covering the positively charged surfaces of magnetic nanoparticles with negatively charged kappa-carrageenan. According to research, the nanoparticles that were synthesized improve the removal of heavy metals and organic contaminants from wastewater. This is accomplished by adsorbing the heavy metals to the surface-active sites of the adsorbent through the electrostatic interaction of the functional groups (Yew et al., 2016; Kulal & Badalamoole, 2020). Therefore, cultivation of seaweed has developed into a significant sector as a means of satisfying the high global demand for the seaweed industry and of compensating for the loss of seaweed populations because of climate change on a global scale.

Kappaphycus salad

Kerabu sare (*Gracilaria* salad)

Noodles made from seaweeds

Agar-agar powder for jelly

Personal care products made from seaweed

Seaweed soap

Seaweed snacks*

3-in-1 seaweed coffee

Figure 1.3 Some available products from *Eucheuma spp, Gracilaria* spp and *Kappaphycus.* *Photo credit: Liu Yung Kuan, Famous Alpine Seaweed Farm

1.4 Seaweed Cultivation Techniques

In general, the seaweed aquaculture sector in the tropics focuses on three of the most commercially important genera: *Eucheuma, Gracilaria*, and *Kappaphycus*. There are different approaches on the cultivation techniques for Eucheumatoids (which include those from the genera *Eucheuma* and *Kappaphycus*) (Figure 1.4) and *Gracilaria* spp.

In the early 1970s, eucheumatoid farming started in southern Philippines and gradually was introduced to the other tropical countries such as Indonesia, Malaysia, the Pacific Island, South Africa, Madagascar and Indo-China (Hurtado & Crithcley, 2006). Cultivation techniques include line cultivation (long line/ monoline cultivation) and floating raft cultivation techniques.

Figure 1.4 Some varieties of *Kappaphycus* culture are harvested from the seaweed farm in Sabah, Malaysia.

1.4.1 Line cultivation (long line/ monoline cultivation)

Basically, line cultivation (long line or monoline cultivation) is carried out in bays or open seas, away from sources of freshwater such as rivers or estuarine areas and other harmful sources such as industrial waste or agricultural waste. The site must also be shielded from large tidal and wind-generated waves to prevent destruction of the farm by these environmental disasters (Pereira & Yarish, 2008).

Selected eucheumatoid seedlings are tied to a short, thinner rope, which is then fastened to the main ropes at sea that range in length from 10 meters to 50 meters, or longer. These ropes are laid out in a parallel manner with variable distances between each other, about 0.5 m to 1.0 m or more, depending on the size of the seaweed that is going to be harvested. This rope, which is stuffed with seaweed, will be positioned at depths that range from those listed below:

a) Off-bottom – The long planting rope will be positioned close to the bottom along the coast, ideally with at least around 0.3 meters of water on top of it at the lowest tide (Figure 1.5). This technique is typically utilized with species that are either small or frequently harvested, such as *Eucheuma* and *Kappaphycus*.

Figure 1.5 Off-bottom seaweeds cultivation model

b) Submerged hanging line - The long planting rope will be positioned in the midwater near to the coast with the condition that it may be submerged in the water during high tides and that it may be at the surface of the water or even exposed during low tides.

c) Long line/floating line - With the support of float balls or aquaculture buoys, the long planting rope will be positioned at or near the surface of the water; meanwhile, the ends of the two ends will be attached and maintained with anchors at the bottom. The cultivated seaweed that is tied along the long rope is kept submerged but is allowed to float relatively close to the seawater's surface (Figure 1.6 and 1.7). With this technique, it is possible to cultivate seaweed regardless of the depth of the seafloor.

Figure 1.6 Long line cultivation system at Semporna, Sabah, Malaysia**.**

Figure 1.7 *Kappaphycus* culture was tied with tie-tie techniques and allowed for vegetative growth along the long line.

1.4.2 Floating raft cultivation

This cultivation technique takes place on the near seawater surface, where the seaweed is attached to lines or nets within a floating rigid frame (Figure 1.8 and 1.9), made of bamboo or some other material.

Figure 1.8 Floating raft cultivation system at Lombok Island, Indonesia.

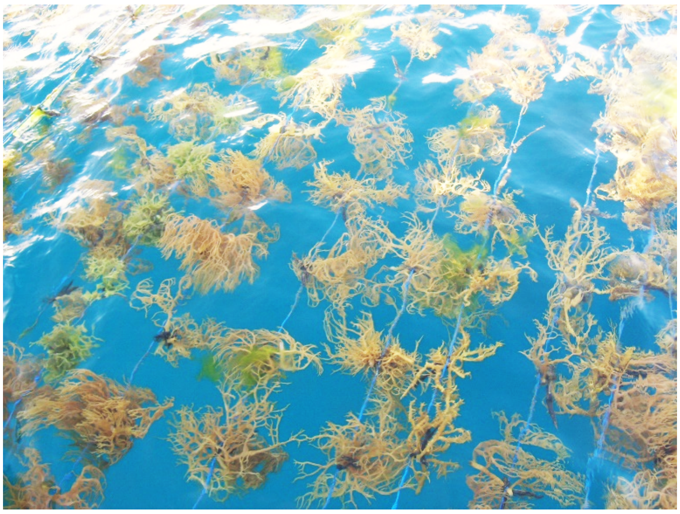

Figure 1.9 The *Kappaphycus* seedlings were attached to lines within a floating raft cultivation system.

Generally, there are four types of *Gracilaria* cultivation methods: the open water cultivation, pond farming, polyculture, and tank cultivation (Critchley, 1993).

1.4.3 Open Water Cultivation

Open water cultivation is typically conducted in bays, estuaries, upwelling locations, and coral flats. Bottom stocking and rope farming are the two techniques utilized in open sea cultivation. Bottom stocking is the simplest way for cultivating *Gracilaria*. This approach involves transferring vegetative thalli that are naturally attached to small stones and shells to the desired cultivation site. At the cultivation site, vegetative thalli will be planted with a fork in soft sediments or affixed to the bottom of cultivation sites using sand-filled plastic tubes. This labor-intensive technique harvests *Gracilaria* when the biomass reaches 15 kg m^{-2}.

Two types of rope farming are available. The first method involves attaching vegetative thalli to a rope and then placing them at the bottom of the cultivation site. The second method of rope cultivation utilizes reproductive elements such as spores. In this procedure, a rope is used to capture the *Gracilaria* spores at their habitat. Then this rope, along with the germlings attached to it, will then be transferred back to the cultivation site for cultivation.

1.4.4 Pond Farming

In China, pond farming is a common practice. Annually, 2000 tonnes of dry *Gracilaria* are harvested from 1500 hectares of ponds (Figure 1.10). Each pond is typically 0.7–1.0 hectares in size and 60–70 cm deep. By regulating the pond water depth, the salinity of the seawater is kept between 10 and 20 ppt, the pH is kept between 7 and 8, and the temperature is kept between 15 and 30 degrees

Celsius. In this technique, ponds are planted with 5–6 tonnes per hectare of *Gracilaria* and supplemented with urea (1 gL^{-1}) and other nutrients. 30–50 percent of the entire biomass is typically gathered for every 30–35 days of cultivation*.* *Gracilaria* is also grown in polyculture systems with economically significant organisms like tilapia, milkfish, prawns, crabs, and abalone.

Figure 1.10 Pond farming cultivation system at Muar, Johor, Malaysia. (Laman Alamjaya Sdn Bhd). Photo credit: Dr Emienour Muzalina Mustafa, UMT.

1.4.5 Tank Farming

Generally, tank farming is a costly type of cultivation. In this technique, the entire process or a portion of the process is automated. As a standard component, an aeration system is crucial for promoting the circulation of seaweed within the tank. In this technique, nutrients like nitrogen-based fertilizer are added. *Gracilaria i*s often picked on a frequent basis to maintain a high level of output (Figure 1.11 and 1.12).

Figure 1.11 Selected seaweed tank cultivation system used for the *Gracilaria* culture, at the hatchery, Universiti Malaya.

Figure 1.12 Growth of *Gracilaria* culture in the tank cultivation system.

1.5 Advanced Techniques of Seaweed Cultivation

Since 1990, the seaweed aquaculture industry has significantly increased its production rate, demonstrating 30 years of steady growth and emergence as a viable industry. It has also become one of the key sources of revenue, particularly for coastal communities in several tropical nations, as an alternative means of subsistence. However, over time, some decline in seaweed productivity has been observed in some countries due to the use of vegetative propagation techniques over a long period of time, resulting in strain fatigue, or loss of vigour (Reddy et al. 2017). To address this issue, some efforts are being made to identify new technologies, one of which is the use of micropropagation techniques to produce high-quality seedlings or plant materials for the industry.

In-vitro cell culture technology has facilitated the development of micropropagation in seaweed cultivation since the year 1978. *Chondrus crispus* and *Laminaria angustata* are seaweed species that accomplished tissue culture in 1978 (Reddy et al. 2008). The initial research on this regeneration study is focusing on the preparation of axenic explants with the fundamental aspects of seaweed tissue culture (Chen & Taylor 1978; Reddy et al. 2003; Yokoya & Yoneshigue-Valentin 2011). Seaweed micropropagation, including protoplasm culture, spore culture, tissue culture, and somatic embryogenesis (Prabowo et al. 2021), is selected to produce disease-free and healthy callus tissues for mass proliferation. Polne-Fuller and Gibor (1987) reported the earliest callus induction by tissue culture studies on *Eucheuma* and *Kappaphycus,* followed by Dawes and Koch (1991), Bradley (1991), Reddy et al. (2003), and Yeong et al (2014). These studies comprised the development of suitable sterilizing procedures for explants, the development of callus induction protocols with suitable induction medium containing plant growth regulators, and the improvement of the growth and regeneration of callus culture by enhancing the culture media. Several following investigations have shown the effective regeneration of micro-propagules directly from explants of *E. serra* (Sahoo et al. 2002), *K. striatus* (Yunque et al. 2011), and *K. alvarezii* (Sahoo et al. 2003, Neves et al. 2015; Yong et al. 2014, Hurtado et al. 2015). As reported by Reddy et al. (2003) and Yeong et al. (2014), these tissue and callus culture techniques provide a clonal propagation method for maintenance of seedstock for seaweed aquaculture, a micropropagation technique for selection of high-quality germplasm and improvement of productivity of economically valuable strains, such as *K. alvarezii*.

As compared to the traditional vegetative propagation, tissue/callus culture and associated micropropagation techniques have provided a strategy for improving the quality and quantity of high-value seedstock and planting materials for the seaweed aquaculture industry. To fulfill the rising worldwide demand for biomass, this high-quality seedstock must be supplied to aquaculture farming in a more effective manner. This may be accomplished using photobioreactors for the purpose of enhancing the growth and productivity of *K. alvarezii* under controlled conditions with important abiotic growth factors such as light irradiance, temperature, mixing time, and the supply of plant growth regulators and nutrients (Muoz et al. 2006 and Yong et al. 2014). These reports have been published in scientific journals. The successful implementation of bioreactors not only provides a scaled-up system for mass production of plant materials for a chosen strain, but also allows for use in overcoming the obstacles of seasonality and environmental variability that hinder the growth of seaweed cultures and in bioprocess engineering production (Rorrer & Cheney, 2004; Reddy et al.2008 and Yong et al. 2014).

In addition to a monoculture approach, the seaweed aquaculture sector might use integrated multitrophic aquaculture (IMTA) to cultivate seaweed alongside fish, shrimp, or mollusks. This IMTA system enables the cultivation of numerous species, including flora and animals, inside a system in which the engaged species mutually benefit one another. The IMTA may be conducted in either an open system (near-shore or at sea) or an enclosed system. In the IMTA model, the co-products (organic and inorganic wastes) of one fauna species are recycled to serve as nutritional input for the other species, and in exchange, the fauna species in the system offer clean water with oxygen to the other fauna species.

The use of IMTA (open system) near-shore or open seaweed farming between or adjacent to fish, shrimp, or mollusk aquaculture was able to mitigate the negative ecological effects of aquaculture while increasing seaweed yield. The land-based enclosed IMTA system with a recirculation aquaculture system and multiple biosensors, as well as controlled culture conditions, was able to optimize the integration of excreted ammonium, phosphates, and CO^2 by seaweeds, and accomplish a cleaning environment for fish and shrimp that offered a more balanced nutrient flow within the system. An important benefit of this method is that it allows the primary farmed species to turn their wastes into additional commercial and environmental value by converting them into sub products like fertilizers, food, and energy instead of aquaculture waste, which enables stronger long-term and overall sustainability and profitability of the culture (Gacía et al., 2020).

The concept of developing a sustainable seaweed cultivation technique is to minimize the negative impact of aquaculture on the environment and at the same time, maximize the product derived from seaweed cultivation. In particular, the seaweed aquaculture industry emphasizes the recovery of optimum biomass of harvested seaweed and then creating a more sustainable ecosystem. Despite this potential, seaweed cultivation acts as a hub for nutrient removal in coastal waters. Scientists are estimating that dissolved inorganic nitrogen going into the coastal ecosystem will escape the shelf break or increase by 2 times by year 2050 compared to year 1990 (Davis et al. 2003; Jickells et al. 2018; Malone and Newton 2020) with one option for recycling of excessive nutrients being in seaweed cultivation technique. While most cultivation technologies only focus on developing a robust and cost-effective system, it is important to note that the advanced technique of seaweed cultivation must be able to improve the condition of coastal waters.

Nutrient bioextraction with seaweed is one of the approaches that can increase the seaweed biomass without utilizing unsustainable nutrients (Park et al. 2021). Through this approach, seaweed cultivation not only supports the food webs and biomass but is also able to improve the quality of coastal waters (Wu et al. 2017; Xiao et al. 2017). It is generally agreed that biosorption or bioremediation using seaweed is one of the most eco-friendly mechanisms for balancing nutrient management and mitigating eutrophication (La Barre et al. 2018; Rubín et al. 2006). To increase the efficiency of the nutrient bio-extraction method, IMTA is implemented as a cost-effective cultivation method while performing the synergistic functions of utilizing the organic and inorganic nutrients in the ecosystem. In the era of Industry 4.0, IMTA, or real-time environmental monitoring or biosensors, through engineering mechanisms, can provide in-situ analysis for better seaweed cultivation planning. However, this data is only able to maintain mainly sustainable seaweed cultivation.

Global climate trends have influenced environmental parameters and the distribution of aquatic diseases, contributing to seaweed's low survival rates. Genetic engineering is applied to manipulate the genes involved in stress tolerance and growth rate to increase the quality and quantity of seaweed. Many investigations have demonstrated the advantages of using molecular tools to study the adaption of tropical seaweed in fluctuating environmental conditions. Gene expressions of seaweed involved in environmental stresses were studied by Nikaido et al. (2000) with *Porphyra yezoensis* using a large expressed sequence tag (EST). After that, different molecular tools such as cDNA microarray, real-time polymerase chain reaction (qPCR) and transcriptomic were used to investigate the defense pathway related to seaweed stress responses, including *Gracilaria changii, Kappaphycus alvarezii*, *Eucheuma cottoniiI* and *Sargussum binderi* (Baharum et al. 2011. Ho et al. 2008; Teo et al. 2009, Smolina et al. 2016; Liesner et al. 2020). Molecular genetic information provides seaweed's environmental tolerance limits and vulnerability to abiotic stresses. With the information provided from the above molecular tools, the molecular priming technique can be applied to generate stress memory in seaweed. This technique is more acceptable than genetically modified organisms (GMOs) and can ensure the production security of seaweed under environmental stresses. However, priming for cultivation still needs to overcome a challenge as mitotically grown generations will reset their genetic memories during meiosis and embryogenesis.

The success of seaweed cultivation will be influenced by internal factors and environmental conditions. Clearly, the factors contributing to enhancing the defense mechanism and level of stress tolerance of seaweed must be investigated through genetic engineering and genetic manipulation approaches. In conjunction with genetic engineering to improve the seaweed strains, multi-disciplinary techniques for seaweed cultivation must be designed to help intensive cultivation of higher biomass seaweed and increase sustainability.

1.6 Conclusion

Since seaweed can produce promising raw materials for food, animal feed, medicine (including nutraceutical and pharmaceutical products), energy (biofuel; biofuel cell), and other uses, there is a need to increase current seaweed production or expand into new seaweed varieties through the development of seaweed cultivation technologies. For the seaweed aquaculture industry, it is essential to maintain research efforts aimed at the development of high-quality seed stocks with properties of disease resistance, rapid growth rate, and a high concentration of desired biochemical compounds, which can be accomplished through micropropagation and bioprocess engineering. New research efforts are also needed in the enhancement of seaweed cultivation techniques, especially with the integration of mechanization technologies for more efficient and eco-friendly aquaculture systems and efficient post-harvest technologies, for the continued growth of the seaweed aquaculture industry. It also ensures the seaweed aquaculture industry's long-term stability and sustainability.

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Chapter 2

ADVANCES IN SHRIMP AQUACULTURE TECHNOLOGY

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ABSTRACT

Scientific applications, technology, and innovations have accelerated the growth of shrimp aquaculture in the past few decades to meet the ever-increasing global protein demands of the expanding human population. Despite challenges and issues associated with worsening environmental conditions, high energy consumption, disease outbreaks, and production losses, the shrimp aquaculture industry is integrating to maximize production by applying shrimp culture technologies that promote the efficient use of time and energy, security, traceability, and sustainability. This article discusses the technologies and applications that have the right properties to address the mentioned challenges, including disease prevention measures and water recirculation from the applied aspects of biotechnology.

KEYWORDS: Shrimp farming, Sustainable aquaculture, Technology, Innovation

2.1 Introduction

Shrimp aquaculture products represent the largest animal protein industry in the consumer market. As crustaceans are nutritious and provide high number of certain nutrients such as iodine that aren't abundant in many other foods, crustaceans are critical in people's lives (Li & Xiang, 2013). According to the Food and Agriculture Organization (FAO) of the United Nations, the world's shrimp production was approximately 8.63 million tons in 2018 and is predicted to increase by 7% (Shinn *et al.*, 2018). The total shrimp production from aquaculture and capture fisheries is assumed to contribute heavily to the global food supply as the human population grows. The world's shrimp production will exceed the poultry and pork world's production in the coming years. Despite the relatively poor knowledge of the cultivated shrimp species and the enormous loss due to disease outbreaks, an estimated approximately 1 billion dollars would be generated every year from the global shrimp consumer market (Li & Xiang, 2013). However, there are many problems and challenges such as disease outbreaks caused by the *Vibrio* pathogen, deterioration of environmental parameters, outdated production systems, and deteriorated water parameters, which could harass the development of shrimp aquaculture and its production. These issues are the main limiting factors in shrimp aquaculture and have resulted in serious economic losses.

Technology and science have benefited almost every aspect of human life in the past decades. The introduction of these technologies and its broadened knowledge over the past years, such as utilizing live feed as aquafeed, disease management technology, including microalgae and zooplankton in hatcheries, have also resolved the bottleneck issues in culturing certain marine species and able to promote the rapid development of aquaculture (Conceição *et al.,* 2010). Although these innovative technologies have contributed to the incredible growth of shrimp aquaculture, the challenges shrimp aquaculture faces are daunting in meeting the expanding demand of the consumer market. For more sustainable and profitable development of shrimp aquaculture, a systemic improvement of existing technologies and the implementation of new technology and innovative feeding methods must make crustaceans' production become more effective, economical, environmentally sustainable, and compliant with governmental regulations. These emerging innovative technologies, including information and automation technologies, recirculating aquaculture systems (RAS), molecular technologies for genetic improvement, biofloc technology, etc., are paving the way toward greater productivity and profitability.

2.2 Common Diseases in Shrimp Farming

Shrimp disease has been a major setback to the shrimp farming industry since the 1980s, when the industry was expanding, and the shrimps were routinely shipped from one region to another. Bacterial diseases caused by *Vibrio* spp. are the major cause of the high mortality rate (up to 90%) in shrimp hatcheries, particularly during early larval stages (Li & Xiang, 2013). Diseases such as Taura Syndrome (TS), White Spot Disease (WSD), and Acute Hepatopancreatic Necrosis Diseases (AHPND) could result in production collapse and cost the shrimp industry billions of dollars in loss in terms of jobs and export revenue (Lightner, 2011). The diseases caused by these pathogens and viruses have been far more problematic to manage as shrimp and the damaging pathogens share the same aquatic environment. The emergence of these shrimp diseases mentioned above results from the complex interactions among the host, the environmental variables, and the surrounding microflora (Xiong *et al.,* 2016). Furthermore, the international movement of live and dead aquaculture products has allowed the transfer of pathogens and diseases from country to country and from one continent to another before the etiology is understood. These diseases in the penaeid shrimp industry could also threaten humans as pathogens and viruses can cross the species barrier (Lightner, 2011).

2.3 Disease Prevention and Management

Due to the lack of adaptive immunity systems in shrimps, classic vaccinations against diseases do not work for them. Although the application of antibiotics could alleviate bacterial diseases, it is not effective against viral diseases. Hence, novel technologies are urgently needed to develop an effective strategy for shrimp disease control.

2.3.1 Vaccination

The immunization of aquaculture started decades ago, and vaccination is an effective method to prevent bacterial and viral diseases. However, as traditional vaccination require each individual to be injected manually, it is considered a labor-intensive process that could cause unnecessary stress to the aquaculture species. Ideally, good vaccine should be safe for the shrimps and provide the host with long-lasting protection (Sivasankar *et al.,* 2017). Hence, immersion and oral administered vaccines as alternative vaccination methods have been studied and developed as they are much easier to handle and require minimal handling skills. This minimizes the damage to the shrimp culture and reduces the mortality rate during vaccination. One of the known oral vaccines in the shrimp aquaculture market is AquaVac™Vibromax™, a V*ibrio* inactivated vaccine manufactured by a UK-based company. It has shown promising results in enhanced growth and survival of shrimp at the post-larvae stage (Amatul-Samahah *et al.,* 2020). However, controlling vibriosis by relying solely on vaccination is unlikely and probable. This should be accompanied by proper sanitization and biosecurity measures.

2.4 Functional Feed from Natural Sources as Alternative Protein Source for Aquafeed

The rapid growth of the aquaculture industry and the ever-increasing demand for farmed marine finfish have resulted in spikes in fishmeal prices over the past few years. The commercial production supply of fishmeal has also faced environmental challenges such as the disruption of nutrition profile, nutritional limitations such as imbalanced amino acid profiles, and the risk of immunological competence with the invasion of pathogens, which eventually lead to disease outbreaks. Hence, the incorporation of different nutritional sources from plants, insects, microorganisms, and natural medicinal products that contain a wide variety of bioactive compounds into shrimp feed has been brought into discussion by researchers and scientists. These innovative sources could maintain the growth and physiological performance and simultaneously enhance shrimps' immunity and disease resistance ability without causing drug resistance.

Innovative feed sources of insect meal and natural medicinal products such as guava (*Psidium guajava*) leaf extract, tiger milk mushroom (*Lignosus rhinocerotis*), black soldier fly (*Hermetia illucens*), and yellow mealworm (*Tenebrio molitor*) (Figure 2.1) contain several types of polysaccharides such as antimicrobial peptides (AMP), β and α-glucans, and chitin, which are potential prebiotics and crucial classes of effector antimicrobial agents (Van Doan *et al.,* 2019; Xia *et al.,* 2021). Compared to current commercial antibiotics that target one specific mode of action, the crude extracts of natural products contain multiple bioactive components that could generate multifaceted responses in bacterial inhibition and are less likely to cause antimicrobial resistance.

Figure 2.1 Leaves of guava (top left); Tiger Milk Mushroom (bottom left); Yellow Mealworms (top right); Black Soldier Flies (bottom right) (Adámková *et al.,* 2017; Alma, 2019; Koe, 2022; Saanvi, 2021)

Single-cell proteins (SCPs) represent alternative feed sources that could be prepared from different microbial sources, including microalgae, yeast, other fungi, and bacteria. SCPs can fulfil the protein needs in the shrimp aquaculture industry as they offer high-quality protein ingredients applicable across species. Feeding trials showed that aquaculture species such as salmon, tilapia, and white-leg shrimp fed with SCPs show improvement in physiological performance (Jones *et al.,* 2020). The study further validated SCP capability to replace fishmeal in aquafeed. Further research regarding the effect of SCPs on pathogenic resistance still needs to be conducted. Despite few publications, these innovative alternative feed sources have great potential to be a nutritious, immune-boost, and sustainable aquafeed that should be tested in the future. These new sources also offer the potential of increasing nutritional value and anti-microbial effects through genetic manipulation (Sánchez-Muros *et al.,* 2014)

2.5 Novel Molecular Technologies for Genetic Improvement

Due to the rapid development of sequencing technologies and the reduction of costs in DNA genotyping, genetic modification or genetic improvement could be the crucial key factors to the rapid development of the shrimp aquaculture industry. The combination of molecular genomic technologies with the traditional breeding method could significantly enhance the genetic improvement of the shrimp species and their immunity capability by increasing their resistance to bacterial and virus diseases. Genetic improvement through genome editing using clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), and zinc finger nucleases (ZFNs) has shown the ability to increase the physiological performance and immunity parameters of aquaculture species (Yang *et al.,* 2022). The application of genome editing allows a quick introduction of desirable alleles into the genomes, increasing the frequency of desired alleles at the loci by determining important traits, generating new alleles, and introducing favorable alleles from other species (Shen & Yue, 2019). Due to the high fecundity and external fertilization of aquaculture species, shrimps are considered suitable for genome editing of many individuals simultaneously (Gratacap *et al.,* 2019). Furthermore, the advancement of high-density single-nucleotide polymorphism (SNP) arrays and routine genotyping by sequencing the selection of desired genome alleles offers a higher selection accuracy than selection based on phenotypic and pedigree records alone (Gratacap *et al.,* 2019). Advances in genome selection and genome editing are dignified to reshape the shrimp aquaculture industry by improving the economically important traits that enable potentially favorable alleles to be introduced into the shrimp breeding program. Hence, subject to favorable regulatory and consumer perceptions, genome selection, and editing technology offers the potential to significantly improve the sustainable production and immunity enhancement of shrimp aquaculture.

2.6 Advances in Modern Shrimp Aquaculture

Although significant growth has been observed in shrimp aquaculture recently, intensification of shrimp aquaculture comes with the cost of unintended consequences such as disease outbreaks and environmental pollution that hinder the development and sustainability of the shrimp industry. As demonstrated by Lightner (2003), biosecurity is the practice of excluding pathogens from the aquaculture culture systems for disease prevention, and it is crucial to the efforts to ensure the productivity and sustainability of the shrimp aquaculture. In the aquaculture system, water quality plays a major role in the health status of the shrimp. Reports have shown that the sudden changes in water temperature, salinity, dissolved oxygen content, toxicity, and eutrophication level could weaken the innate immunity of shrimp and convert opportunistic pathogens from non-virulent to virulent, leading to poor growth and disease outbreaks (Boutin *et al.*, 2013). With the fluctuating water quality conditions brought about by rainy seasons in Southeast Asian countries, shrimp farmers are shifting to farming models aimed at achieving zero water exchange to keep the shrimp culture environment fully controlled and kept away from disease outbreaks caused by bacteria, viruses, and pathogens. A reduction in water exchange ensures biosecurity by preventing the entry of disease carriers from external sources and promoting environmental sustainability by reducing land and water requirements (Chamberlain, 2005).

2.7 Recirculating Aquaculture Systems

Recirculating Aquaculture System (RAS) has been widely adopted since the 1980s by shrimp farmers, especially those who practice intensive culture to achieve stable water quality parameters. RAS is a technology that involves multi-step treatment of water, including solids removal, filtration, denitrification, and disinfection to make it suitable for reuse in the culture system of aquatic animals (Figure 2.2). The recirculation system uses biofiltration and oxygenation to improve water quality and productivity.

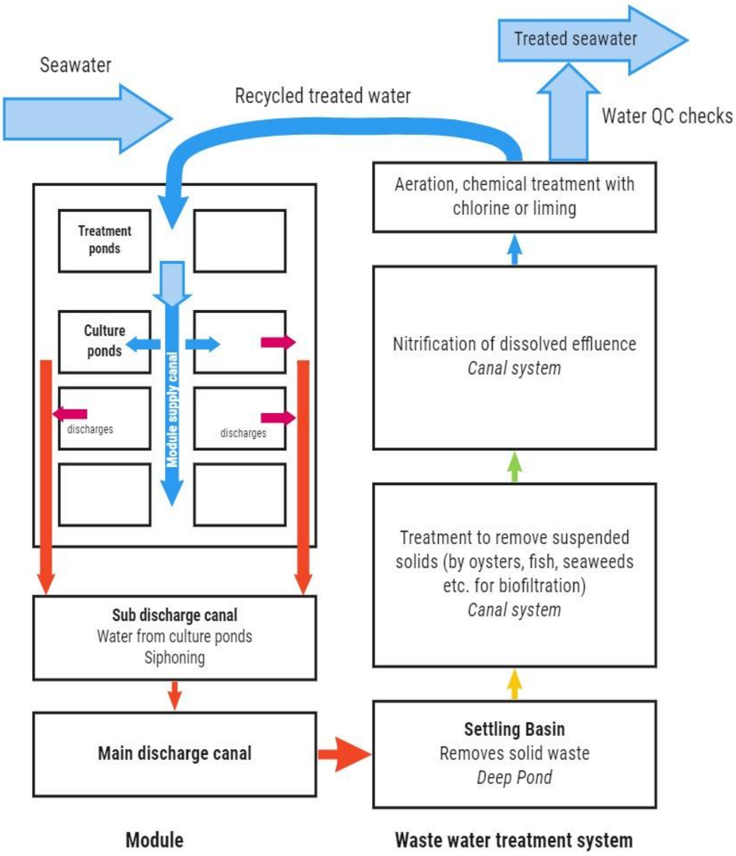

Figure 2.2 Recirculating Aquaculture System (RAS) within modules described by Taw *et al.,* (2013)

Cultivating shrimp with reduced water exchange is beneficial, especially in certain regions where water is a limited resource (Chamberlain, 2005). RAS is considered one of the most environmentally friendly ways of producing shrimp at a commercially viable level. The benefits of RAS aquaculture in terms of efficiency and sustainability have prompted Charoen Pokphand Foods (CP Foods), the Thai food giant's decision to move all its shrimp farms indoors (Holmyard, 2019). Despite the major issues such as the high investment needed for constructing facilities, logistics and staff upskilling, RAS will be a long-term solution for the shrimp farming industry, enabling better yield, disease control and quality. The large investment budget will have a positive impact on both production and environmental sustainability and eventually outweigh the costs eventually.

2.8 Biofloc Technology

Biofloc technology (BFT) is another efficient and environmentally friendly method to produce shrimp with relatively low operational costs. Biofloc systems maintain water quality by using microbial communities that continuously recycle and reuse nutrients in the environment. Microorganisms are capable of up-taking and converting wastes in the form of nitrogenous metabolites into microbial proteins that serve as a useful source of dietary proteins for cultured shrimps (Nisar *et al.,* 2022). Originally conceived as a low-cost natural means of clean water, biofloc systems are becoming increasingly popular as they provide an additional carbon source to the aquaculture system, improving feed ingestion and digestion while reducing the feed conversion ratio (Chamberlain, 2005).

Significant infectious disease outbreaks have prompted the implementation of strict biosecurity measures, such as close monitoring of the shrimp culture environment and reducing water exchange. Various findings have proven the ability of the BFT to control unfavorable chemicals and toxic substances in aquaculture systems. Production intensity in BFT systems is revealed to be higher than in non-BFT culture systems (Nisar *et al.,* 2022). Nowadays, creative and inventive shrimp-industry players are creating and implementing BFT in conjunction with other advances to achieve more productive integrated systems that produce more food with less input and resources. Important principles and components from RAS and biofloc technology are being combined to ensure and enhance the scalable efficiency.

More experimentation and research must provide more accurate data regarding the appropriate conditions for different species and carbon sources in the aquaculture system. Shrimp farmers must be trained in the practical application of the BFT to ensure the effective use of biofloc systems, enabling a natural way of producing more seafood sustainably while concurrently improving farm profitability.

2.9 Information/Digital Technologies

The technology of Internet of Things (IoT) allows quick collection and analysis of massive amounts of streaming data across the entire shrimp aquaculture industry. Although IoT is relatively new to the shrimp aquaculture industry, these technologies are proven to bring huge benefits to the sector. With the application of information technology and IoT, the environmental conditions in shrimp breeding sites could be efficiently observed in real-time and with higher coverage by incorporating underwater cameras and sensors across multiple breeding farms. Furthermore, IoT allows real-time collection and analysis of environmental and water parameters conditions and provides contextual information to ensure the quality of the breeding cage (Jothiswaran *et al.,* 2020). The combination of IoT (Figure 2.3) and robotics can revolutionize shrimp aquaculture in many aspects as it would make the most labor-intensive and risky tasks such as feeding, pond, and net cleaning, behavior monitoring, and sick fish removal be done much more efficiently at a rather low cost (Kamaruidzaman & Rahmat, 2020). The application of automation technology could make the shrimp industry more profitable as robotics can operate continuously without any interruption under difficult conditions and without the need for human assistance (Yue & Shen, 2021). Certainly, introducing automation and information technology would bring substantial changes to the shrimp aquaculture industry. These technologies must be properly applied to the field in the future.

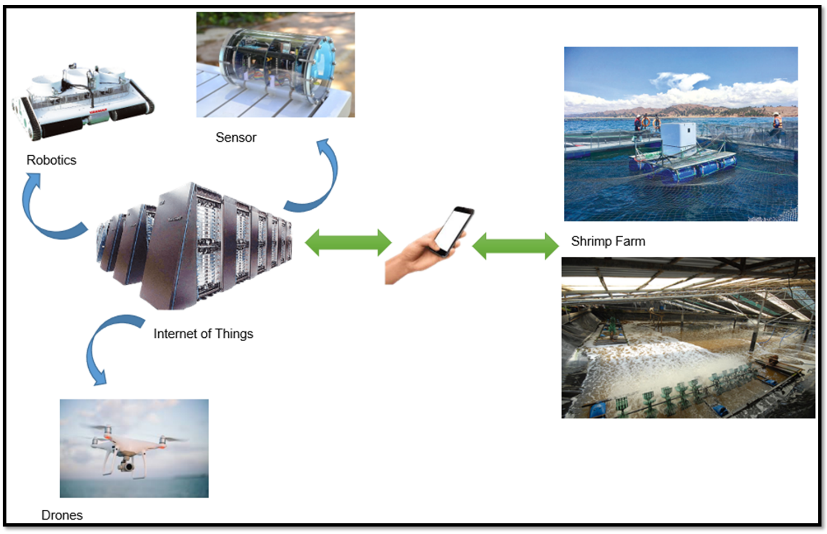

Figure 2.3 The emerging information/digital technologies, including robotics, drones, sensors, artificial intelligence (AI), etc. are applications that increase the shrimp aquaculture production. These technologies are connected with shrimp farms through satellites, the internet of things (IoT), and mobile phones.

2.10 Conclusion

Shrimp aquaculture plays a crucial role in supplying good quality protein to the high-demand consumer market. The requirement for shrimp supplies is expected to increase substantially in the coming years due to the ever-expanding human population. Cutting-edge and novel technologies must address the main challenges such as high production costs and high disease incidences with low survival rates. In the past few decades, the shrimp industry has encountered major setbacks from disease outbreaks, as well as water and land resource challenges. As the industry has grown, so too have inventions and technological advances. Technologies and methods such as innovative feed sources, genome editing, information technologies, recirculating aquaculture systems, and biofloc technology have shown potential in revolutionizing the shrimp industry by offering more sustainable, predictable, and profitable aquaculture. The industry is consolidating, and shrimp farmers are integrating technologies, demonstrating continuous improvement in shrimp farming techniques. With the right approaches and management to apply the available resources effectively, the shrimp industry can achieve a secure and consistent shrimp supply chain.

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Chapter 3

COPEPODS AS LIVE FEED FOR MARINE LARVICULTURE: ADVANTAGES, CHALLENGES AND FUTURE PERSPECTIVES

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ABSTRACT

With the current trend in human population growth and overexploitation of wild fish, aquaculture is the best solution to support the declining fish production worldwide. Although the overall fish production from aquaculture has exceeded the wild fish production in the last few years, it is still unlikely that the marine finfish from aquaculture can surpass the wild production soon. The success of seed production in mariculture is highly dependent on the type of feed given particularly at the first feeding stage of marine fish larvae. While inert feeds progressively improved in nutritional composition, live feeds are still preferred for marine fish larvae rearing since they are natural food to fish larvae. Aquaculture live feeds consists of microalgae, *Artemia*, cladocerans, copepods and rotifers. Among these, copepods constitute the most ideal feed that meet almost all nutritional requirements of marine fish larvae. High nutritional composition (e.g. DHA, free amino acids, pigments and vitamins), swimming behavior and size of copepods are advantages that allow optimal growth of fish larvae. No enrichment is required for copepods, unlike *Artemia* and rotifers with some nutrient deficiencies. Unstable production and expensive microalgal feeds are major constraints that hinder copepods as feeds in large scale aquaculture system. There has been a progressive improvement in copepod culture at commercial scale including the establishment of diapause egg

product and alternative feeds of bacteria and ciliates which can be potentially more cost-effective than microalgae in copepod culture. However, more works on innovation and optimization are still needed if commercialization of copepod live feeds is intended. This chapter aims to review and discuss the biological features, advantages, challenges and future perspectives of copepods as live feeds in marine larviculture. The status and potential of copepod culture based on the Malaysian scenario are also highlighted.

KEYWORDS: Copepods, fish larvae, live feeds, mariculture, nutritional composition

3.1 Introduction

With the increased pressure on global food supply due to the exponential growth of world's human population and its direct and indirect impacts on environment, climate and political status, food security is now the priority of almost all country's policy. The sufficient food supply would ensure the political stability and sustainable development of a country. Therefore, the novel technology is crucial to improve the quality and yield of agricultural products as to meet the pace of human population growth. Agriculture not only involves the production of terrestrial crops and poultry but also farming of aquatic organisms or commonly known as aquaculture. In comparison with other agricultural sectors, aquaculture has experienced the most rapid growth of production in the last two decades. To date, more than half of the global seafood production come from aquaculture and this contribution is expected to increase further in future (FAO, 2020). Even it is unlikely that the production of marine farmed fish can exceed the production of capture fisheries in the near future, mariculture is still the best solution to ease the existing pressure on the overexploited wild fish in ocean.

It is more challenging to culture marine fishes as compared to their freshwater counterparts due to the complex environmental set up and nutritional requirements especially for young larval stages. The first larval feeding is the most critical stage that would ensure the success of seed production in aquaculture. With the incomplete development of digestive system, the specific food criteria are required for optimal growth and survival of fish larvae (Infante & Cahu, 2001; Yúfera & Darias 2007; Hu et al., 2018). As the natural food in the wild, the planktonic organisms such as microalgae, *Artemia*, ciliates, cladocerans, copepods and rotifers are commonly used as live feeds for marine larviculture (Pan et al. 2022). In most cases, the unsuccessful seed production of marine fish is mainly attributed to unstable live feed supply during the critical stage of first larval feeding (Santosh et al., 2018). Several attempts have been made to replace live feeds with inert feeds but the results were not as good as live feeds for marine larviculture (Chen et al., 2006). The inert feeds are generally poor in palatability and quality. This is not suitable for fish larvae with incomplete development of digestive system (Infante & Cahu, 2001; Yúfera & Darias, 2007). Furthermore, the inert feeds tend to aggregate and sink to the bottom, making the feeds not only less accessible to the fish larvae but the uneaten food would also deteriorate the water quality of the rearing tank. As opposed to inert feeds, the planktonic live feeds are more visible and available to the larvae since they drift in the water column. The movement of planktonic live feeds can also stimulate the hunting of fish larvae as a visual predator and thus initiate feeding of the fish larvae (Conceição et al., 2010; Nielsen et al., 2017; Kandathil et al., 2020).

In order to meet the demand of large-scale larval rearing, live feed must have some basic advantages such as short life cycle, high reproductive rate, hardy, nutritious, optimal size and unique swimming behaviour (Drillet et al., 2011). The common live feeds cultured for marine fish larvae rearing include *Artemia*, copepods and rotifers. Among these animals, copepods constitute the most ideal feed to marine fish larvae since they are the natural prey to most of the fish larvae. This can be supported by the fact that more than 90% of the larval fish diets is predominated by copepods even though there are diverse groups of zooplankton available in marine ecosystems (Sampey et al., 2007; Llopiz, 2013; Quah et al., 2022). The brackish copepod species *Pseudodiaptomus annandalei* was consumed by many juvenile fishes in tropical mangrove estuaries before the ontogenetic shift to macrobenthic diets (Chew et al., 2012). In view of its significance to small fish diets, *P. annandalei* was isolated and cultured as live feed for grouper larvae (Liao et al., 2001; Chen et al., 2006).

Although the efficacy of copepods as live feeds to marine fish larvae has been well acknowledged, the stable and reliable stock of copepods is a key challenge in aquaculture industry. There have been about 60 copepod species successfully grown in captivity since 1960s and the first large scale production was established in 1980s for the euryhaline species *Acartia tonsa* in Denmark. Since then, several dominant species in temperate waters (e.g. *Acartia* spp., *Centropages* spp., *Eurytemora* spp. and *Oithona* spp.) have also been raised (Drillet et al., 2011). In recent years, a total of nine common copepod species from the families Acartiidae, Euterpinidae, Oithonidae, Paracalanidae, Pseudodiaptomidae and Temoridae have been successfully cultured and used for marine larval fish rearing in India (Santosh et al., 2018). Despite the progressing success over time in copepod culture techniques, the production of reliable copepod culture on large scale remains the main challenge (Dhont et al., 2012). This chapter aims to review and discuss the biological features, advantages, challenges and future perspectives of copepods as live feeds in larviculture of marine fish larvae. The status and potential of copepod culture based on the Malaysian scenario are also highlighted.

3.2 Why Copepods?

In comparisons with other animal live feeds such as *Artemia* and rotifers, copepods constitute the most reliable feed to marine fish larvae in terms of nutritional value, size and physical behaviour (Drillet et al., 2011; Santhanam et al., 2019; Burbano et al., 2020). As the most abundant metazoan on Earth, copepods are important intermediaries transferring the energy

source from the base of the food webs to top predators. Many studies on the larval fish diets in marine ecosystems show the high preference of fish larvae towards copepods over other zooplankton taxa (tunicates, various meroplankton) as prey (Sampey et al., 2007; Llopiz 2013; Quah et al., 2022). The plunge of copepod wild stocks as attributed by anthropogenic impacts had significantly impinged the production of capture fisheries in Atlantic waters (Beaugrand et al., 2003). In mariculture, the fish larvae perform better in terms of growth and survival when feeding them with copepods compared with *Artemia* and rotifers (Shields et al., 1999; Hamre et al., 2002; Rajkumar & Vasagam 2006). Like the wild environments, the low supply of copepod live feeds during the critical larval feeding period in mariculture had led to poor development and survival of fish larvae (Støttrup et al., 1998; Hamre et al., 2005; Busch et al. 2011). The high selectivity on copepods over other zooplankton taxa as food by fish larvae is mainly due to the high nutritional value of copepods particularly the polyunsaturated fatty acids (PUFA) that are essential for marine larval fish growth (Shields et al., 1999; Hamre et al., 2005; Busch et al., 2011; Matsui et al., 2021).

Based on biochemical profiling, copepods appear to have higher unsaturated fatty acids (n-3 HUFA) than that of *Artemia* and rotifers (Kanazawa 1993; Reitan et al., 1994; Bell et al., 2003). Although with the moderate lipid contents, high DHA relative to EPA and ARA (>20) in copepods are the key factor that allows the optimal development and growth of marine fish larvae (Bell et al., 2003; van der Meeren et al., 2008). In contrast, the minimal DHA/EPA/ARA ratios required for normal larval fish growth can only be achieved in *Artemia* and rotifer cultures via HUFA enrichment. However, the assimilation of essential fatty acids by the larval fishes from the enriched *Artemia* and rotifers is still less efficient as compared to copepods. HUFA is located in the phospholipid fraction of copepods while the enriched *Artemia* and rotifers are located in the neutral lipid or triacylglycerol fraction (Coutteau & Mourente 1997; Bell et al. 2003). As HUFA in the phospholipid faction are more accessible and digestible than that in the triacylglycerol fraction, this conforms the nutritional advantages of copepods over other live feeds in marine larviculture (Izquierdo et al., 2000; Gisbert et al., 2005).

Besides HUFA, copepods also consist of higher free amino acids (FAA), astaxanthin and iodine as compared to *Artemia* and rotifers (van der Meeren et al., 2008). The free amino acids are crucial for protein uptake and encourage a better growth performance during the critical first-feeding stage (Fyhn et al., 1993; Rønnestad et al., 2003; Rønnestad & Conceição 2005). The astaxanthin pigments are the source of antioxidants and vitamins while the iodine is the precursor of thyroid hormones. The lack of astaxanthin and iodine during the developmental period would lead to malpigmentation and abnormal growth of marine fish larvae (Shields et al.,1999). With their high nutritional value especially the HUFA, enrichment of copepod live feeds is normally unnecessary, unlike *Artemia* and rotifers with some nutrient deficiencies.

3.3 Copepod Species as Live Feed in Aquaculture

Copepods are the most abundant and diverse metazoans on Earth which consist of 10 orders and can be found in almost all aquatic environments (Razouls et al., 2022). However, not all copepod species are suitable as live feeds as some species are parasitic while others are carnivorous. Most of the cultured species are planktonic and belong to the three orders, namely Calanoida, Cyclopoida and Harpacticoida. The calanoids constitute the largest group in marine environments. The genera that have been well studied include *Acartia*, *Calanus*, *Centropages, Paracalanus*, *Pseudocalanus* and *Temora* (Mauchline 1998). Among these, species from the genera *Acartia*, *Centropages* and *Eurytemora* have been successfully cultured in Europe (Støttrup, 2003). In Asia-Pacific region, the genera that have been successfully raised for larval fish rearing include *Acartia*, *Bestiolina*, *Paracalanus*, *Parvocalanus*, *Pseudodiaptomus* and *Temora* (McKinnon et al., 2003; Santosh et al., 2018). The advantages of some calanoid species as live feeds include their high nutritional value and the diapause eggs that can be stored and revived when desire (Marcus & Murray 2001; Drillet et al., 2007). The major drawback of calanoid culture is its low tolerance to crowding that may lead to low hatching and survival rates (Peck & Holste 2006; Jepsen et al., 2007). For some omnivorous towards carnivorous species such as *Acartia* and *Centropages*, cannibalism among own species is high and therefore prevents high density culture (Ohno & Okamura, 1988).

The cyclopoids cover a broader range of distribution, living not only as benthic and planktonic forms but also as parasites. The non-parasitic cyclopoids are mostly omnivorous or detrivorous that feed on organic particles from their environments. Most of the harpacticoids are benthic and feed on bacteria from the biofilms or organic detritus. The few common planktonic species of harpacticoids are *Euterpina acutifrons*, *Clytemnestra scutellata* and *Microsetella norvegica*. The advantages of cyclopoids and harpacticoids as live feeds in aquaculture include their broad dietary spectrum other than microalgae, high tolerance to poor water quality environments and thus increase the survival rate, ability to co-exist with the invasive organisms such as ciliates, nematodes and rotifers (Cutts, 2002; Rhodes, 2003; Støttrup, 2003), high reproductive rate and resilient to crowding (Støttrup, 2003). The major drawbacks are their lower nutritional value compared to calanoids and benthic behaviour that are less accessible by planktonic fish larvae. Thus, the planktonic species such as *Oithona* spp. and *E. acutifrons* are more ideal for fish larviculture.

3.4 Culture Methods and Challenges

Similar to other live feeds, copepods can be cultured using extensive, semi-intensive and intensive methods. The extensive and semi-intensive methods are dependent on the source directly or indirectly from the natural environments. Copepods are obtained by filtering the natural seawaters through a certain mesh size sieve. The pitfalls of this method are the introduction of composite organisms including pathogens and predators into the culture system. The high-density copepod culture and low reliance on the well-trained workers can be achieved by intensive and automated culture system (Rhodes, 2003; Støttrup, 2003).

The key success of marine larviculture is highly dependent on the quality of feeds especially at the first feeding stage. Low seed production and abnormal development of larval fishes are always associated with their poor dietary contents in HUFA, free amino acids, vitamins and some essential nutrients (Støttrup et al., 1998; Hamre et al., 2005; Busch et al., 2011). Although copepods are generally accepted as the universal feeds to marine larval fishes, it is challenging to culture copepods at large scale. There is a risk to introduce pathogens, invasive species or predators to larval fish culture if the seawater is directly obtained from natural environments (Støttrup, 2003; Dhont et al., 2013). The most common issue encountered in copepod culture is its tendency to be infested by stalked ciliates if seawater is not properly treated. The coexisting nematodes and rotifers may outcompete copepods in terms of food and space. Meanwhile, the voracious predators such as ctenophores and chaetognaths can prey on both naupliar and adult copepods. The herbivorous copepods are mainly fed with diatoms which require the special medium (e.g. F_2) for proliferation and regular sub-culture to avoid culture stock crash. The use of cheap and readily available feed like flour and yeasts is not advisable since these substances would deteriorate the water quality of culture. The copepod culture itself requires regular water quality maintenance, stock harvesting and sorting. Although some hatcheries have successfully established the large-scale culture, the process from microalgal food production to copepod culture maintenance is expensive and labour intensive (Santosh et al., 2018).

3.5 Future Perspectives

The commercial copepod live feed products are less common in the market as compared to other aquatic live feeds such as microalgae, *Artermia*, cladocerans and rotifers. Almost all copepod live feed products available in the market are either frozen or preserved, and used mainly for ornamental purpose. This is normally expensive and economically infeasible for large scale aquaculture production. Although the copepod diapause eggs have been successfully produced by an aquaculture product company (i.e. CFEED) in recent years, the initiation of copepod population from diapause eggs is still not user friendly as the cysts from *Artemia* and rotifers. In most cases, copepods are used to feed only the critical early larval stages and replaced with *Artemia* and rotifers for the older stages. There has been almost no report on marine larviculture that exclusively relies on copepods as live feed. This scenario is a result of the unstable copepod production for large scale aquaculture system. Therefore, more developments, innovations and optimization of culture techniques are needed to ease the issues of unsustainable production as well as storage and logistics for copepod live feed products.

To achieve the commercial status like *Artemia* and rotifers, the innovative techniques of how to mass produce viable copepod eggs that can be stored and revived when desired, stands a great potential in marine larviculture. The attempts to preserve and prolong shelf-life of copepod eggs (Drillet et al., 2007) can be further researched to optimize and enhance the copepod live feed reliability. Copepods do not produce cysts that can be harvested and dry-packed, unlike *Artemia* and rotifers. Researchers and live feed producers can adopt the similar concept by converting the copepod diapause eggs or egg sacs to the commercial products that can be stored and used like *Artemia* and rotifer cysts.

Since the production of microalgal feeds is expensive and labour intensive, the development of alternative feeds such as bacteria not only can reduce the operational cost but also as a backup when there is crash in microalgal culture. The addition of commercial probiotics was reported to significantly improve the egg production and hatching success in *A. tonsa* cultures (Drillet et al., 2011), indicating that bacteria can be potentially used in copepod culture. It has been found that rotifers fed phototrophic bacteria that grown in palm oil mill effluent (POME-Bac) have higher PUFA than the population that was cultured with microalgae (Loo et al., 2015). Although there has been no published work on the use of POME-*bac* as a feed for copepods, the similar research team has successfully maintained the calanoid culture in their hatcheries. As the main palm oil producer in the world, the conversion of POME to bacteria feed for copepod culture constitutes a great potential of aquaculture industry in Malaysia.

Although the importance of copepods in marine larviculture is generally accepted by the local farmers, most of the marine fish farms in the county are still depending on *Artemia* due to its readily available stock in the market. There are few dominant copepod families found in both brackish and marine waters of Malaysia such as Paracalanidae, Pseudodiaptomidae, Oithonidae and Euterpinidae (Chew & Chong 2011; 2016). These families constitute the main diets of both larval and juvenile fishes in Malaysian waters (Chew et al., 2012; Quah et al., 2022). However, almost none of them are used as live feeds in aquaculture industry in Malaysia. Most of the copepod culture works in the country were carried out by research institutions or universities. The species involved were from the families Acartiidae and Oithonidae, while the feeds used were conventional microalgal feeds and baker's yeasts (Rajkumar & Rahman 2016; Rasdi et al., 2021). The outputs might be doable at laboratory-scale but more advance techniques are required if commercial scale is intended. Besides Acartiidae and Oithonidae, future research can be extended to other ecologically important families such as Paracalanidae, Pseudodiaptomidae and Euterpinidae, while the feeding trials with other alternative feeds (e.g. bacteria and ciliates) can be considered as a replacement or supplement for microalgal feeds. The collaboration between academic researchers and industrial partners is highly recommended to tackle the exact needs and issues of the end users and establish the commercial copepod live feeds that are effective and user friendly to aquaculture industry.

3.6 Conclusion

With both field and laboratory studies, copepods have been proven to be the most optimal food for marine fish larvae. Despite diverse zooplankton groups in marine ecosystems, only a few dominant copepod species (i.e. *Parvocalanus crassirostris*, *Pseudodiaptomus annandalei*, *Oithona* spp. and *Euterpina acutifrons*) predominate the dietary composition of fish larvae. While these species are abundantly found in natural environments, their proliferation in captivity and application on commercial scale remain a challenge. So far, the diapause eggs of *Acartia tonsa* are developed into the commercial product. However, the *Acartia* species may not be a good candidate as the universal food since most of the fish larvae in tropical waters prefer other copepod preys over *Acartia* (Quah et al. 2022). If the same techniques can be adopted and optimized for the more universal species stated above, there would be a great achievement to resolve the issue of copepod live feed supply that have existed for a long time. With the ongoing pace of research and development on copepod live feeds, the commercialization of copepod live feeds in aquaculture industry will be realized in the near future.

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Chapter 4

THE IMMUNOMODULATORY EFFECTS OF INSECT MEAL IN AQUACULTURE

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ABSTRACT

Dietary supplement of immunostimulants to maintain fish health and enhance disease resistance has been widely used worldwide. Several insect species have been found to contain a broad range of bioactive substances of optimising fish health. The most studied and applied is Black Soldier Fly (*Hermetia illucens*). The flies are easy to grow and reproduce, full of nutrition, have high feed efficiency and low rearing pollution. It is rich in chitin, antibacterial peptide and lauric acid which adds the potential as an immunostimulant and health promoter of fish culture.

KEYWORDS: black soldier fly, immunostimulant, fish culture

4.1 Introduction

Black soldier fly (BSF) is an important alternative protein source recognized by the Food and Agriculture Organization (FAO) to maintain sustainable economic and environmental development (Nogales et al., 2019). Meanwhile, BSF is rich in bioactive compounds, which can effectively improve the immune state of aquatic animals (Gasco et al., 2021; 2018; Mousavi et al., 2020). There are numerous findings on the immunostimulatory effects of dietary black soldier fly on terrestrial animals. However, the findings in aquaculture are still at an early stage (Mousavi et al., 2020). To study the immune enhancement effect and health level of BSF substitution in various fish species is a hot research question in aquaculture in recent five years.

4.2 Atlantic salmon (*Salmo salar*)

In 2018, the United States approved BSF to be used as food in salmonids (AAFCO, 2019). Few studies about Atlantic salmon have been performed (Li et al. 2021; 2020; 2019). They found that total replacement of fish meal (FM) with 15% inclusion of BSF larvae meal in diet after 8 until 16 weeks did not compromise the gut health of the fish, but to some extent it seemed to affect positively. BSF meal diet induced the same level of infectious pancreatic necrosis virus (IPNV) antibody, immune and barrier-function gene expression profiles when compared to control group with other immunostimulants. Steatosis of enterocytes was observed in the fore and mid intestine in BSF meal diet group as well. BSF group increased the expression of genes related to stress response, immune tolerance and detoxification activity; a higher relative weight of distal intestine was found in fish fed with the BSF meal diet. Meanwhile, BSF fed fish showed less lipid accumulation in enterocytes based on the degree of hyper-vacuolization revealed by histological examination of the pyloric caeca mucosa. BSF supplemented diet also increased microbial richness and diversity of digesta- and mucosa-related gut microbiota. On another hand, Weththasinghe (2021) compared the effects of BSF larvae meal and paste substituting conventional protein sources in Atlantic salmon for 7 weeks on gut health, skin mucus proteome and immune reaction. The results were like the above, showing that BSF had small effects on skin mucus proteome as well as immune reaction. But low to moderate levels of larvae meal with 6.25% and 12.5% or paste with 3.7% and 6.7%, BSF replacement decreased enterocyte steatosis in pyloric caeca. While replacing level up to 25% with larvae meal or 6.7% with paste, BSF improved distal intestine histology. The plasma C-reactive protein linearly decreased with increasing BSF larvae meal level, while the plasma antioxidant capacity linearly increased with increasing BSF larvae paste level.

4.3 Rainbow trout (*Oncorhynchus mykiss*)

For rainbow trout, Huyben (2019), Terova (2019), Rimoldi (2019) studied the effects of partially defatted, fully defatted or full-fat BSF larvae or prepupae meal as substitutes for FM on gut microbiota of the fish for 5 to 12 weeks. The results were almost identical and showed that up to 50% replacement FM with 30% inclusion of BSF had positive effect on gut microbiota of the fish. BSF diets positively modified gut microbial composition, increasing its richness and diversity. For example, after feeding with BSF-based diets, the amount of lactic acid- and butyrate-breeding bacteria increased; the abundance of *Firmicutes* and *Actinobacteria* increased while *Proteobacteria* reduced; the abundance of *Corynebacterium* increased, which was attributed to the production of lipase and the high levels of dietary lipids; the abundance of *Bacillaceae* and *Mycoplasma* significantly increased, which was attributed to the production of chitinase and the high content of dietary chitin. Terova (2020) studied effects of partially defatted BSF larvae meal substituting FM on hepatic methionine (Met) metabolism. The results of 11-week feeding trial indicated that up to 50% of FM replacement with BSF did not negatively affect hepatic Met metabolism in rainbow trout. Particularly, Met availability in the BSF groups directly regulated the transcription levels of two target genes (CBS, cystathionine β synthase which participate in net Met loss; SAHH, S-adenosylhomocysteine hydrolase which is involved in Met resynthesis) in the Met pathway, keeping an optimal concentration of one-carbon metabolic substrates, i.e., SAM (S-adenosylmethionine): SAH (S-adenosylhomocysteine) ratio in liver tissue. Kumar (2021) found that supplementation of BSF larvae effectively prevented intestinal enteritis induced by soybean meal and using BSF oil is also beneficial to the health of rainbow trout. After 10-week feeding trial, BSF larvae supplementation of 16% down-regulated the prostaglandin and interferon regulatory factor 1 (IRF-1) level in gut, while significantly increased serum lysozyme activity; BSF oil caused no hepatic or intestinal histomorphological change, but tumor necrosis factor, interluekin-8 and IRF-1 in kidney were significantly upregulated along with serum peroxidase activity was significantly increased; the inclusion of bile acid in the BSF oil group significantly upregulated the gene expression of intestinal prostaglandin.

4.4 Bass

There have been reports on feeding BSF meal to largemouth bass (*Micropterus salmoides*), Asian seabass (*Lates calcarifer*), European seabass (*Dicentrarchus labrax*). Several literatures show that appropriate supplementation of BSF meal to the basal diet could improve blood and serum biochemical response, promote health of intestine and liver, enhance the antioxidant activity, decrease oxidative metabolites and down-regulate relative gene expression, up-regulate the immune gene expression, increase disease resistance of bass.

4.5 Largemouth bass (*Micropterus salmoides*)

Fischer (2022) performed an 8-week feeding trial to evaluate effects of full-fat BSF larvae or pre-pupae dietary supplementation in juvenile largemouth bass. It was reported that most of the hepatic/intestinal gene expression in the 11.9% BSF larvae or/and 9.83% BSF pre-pupae treatments generally mirrored those of the fishmeal-based or/and soybean meal-based control diet. The products of these gene expressions are related to growth, lipid metabolism, antioxidant sentinels and inflammatory cytokines, such as insulin-like growth factor (IGF-I) and growth hormone (GH), cholesterol 7 alpha-hydroxylase (CYP7A1) and fatty acid synthase (FASN), superoxide dismutase (SOD), tumor necrosis factor-α (TNF-α) and transforming growth factor- β1 (TGF-β1). But intestinal FASN and hepatic TGF-β1 expression in 9.83% BSF pre-pupae group were down-regulated compared to other groups. However, liver and intestinal histopathology reveal that BSF larvae is better for the fish compared to BSF pre-pupae.

Peng et al. (2021a; 2021b) conducted a 9-week feeding trial to assess dietary BSF pulp on liver and intestine histomorphology, liver lipid metabolism gene expression, antioxidant and immune capacity of largemouth bass. Adding inclusion of 9% BSF pulp in diets had optimal immunostimulatory effects to the fish, considering effect on growth performance meanwhile. Because in this treatment, serum malonaldehyde (MDA) was reduced but acid phosphatase (ACP) was raised, liver peroxisome proliferators-activated receptors α (PPARα) and lipoprotein lipase (LPL) mRNA down expressed. And in BSF pulp treated diets, intraperitoneal fat deposition was increased, hepatocytes accumulate more lipids accompanied by certain degrees of hyper-vacuolization and inflammatory cell infiltration.

He suggested BSF pulp should be careful to be used in largemouth bass diets. Xu (2021) performed the bacterial challenge experiments with *Aeromonas hydrophila* to largemouth bass fed for 8 weeks. The results indicated that the cumulative mortality rate after 72 h in 1% zymolytic BSF pulp group significantly dropped, while the 5% zymolytic BSF pulp group sharply accelerated. Meanwhile, antioxidant activity increased, and oxidative metabolites reduced in 1% zymolytic BSF pulp group. Furthermore, compared with 0% group, great changes took place in the intestinal microbial composition of 1% group. The dominant phylum Tenericutes changed into Fusobacteria, and the most abundant genus *Cetobacterium* of Fusobacteria replaced *Mycoplasma* of Tenericutes. The results at the level of microbial potential function showed that amino acids, energy and nucleotides metabolism increased, while glycans and lipids metabolism decreased, and α-N-acetylglucosaminidase activity improved notably. Briefly, 1% zymolytic BSF pulp or 9% BSF pulp or 10% BSF pupae or 12% BSF larvae replacing FM for 8-9 weeks was appropriate to largemouth bass. However, it should be noted that excessive addition may cause side effects.

4.6 Asian seabass (*Lates calcarifer*)

Chaklader (2021), Gupta (2020), Hender (2021) were unanimous that BSF diet could be a promising protein source substituting FM in the diets of Asian seabass. But the immunostimulatory effects of dietary BSF on the fish varied quite widely in the above research, the proposed optimal replacement proportion was inconsistent. One of the aspects that contributes to this inconsistency is the feeding formula which was different. Chaklader agreed that FM protein could be totally replaced by the ingredients, containing 30% full-fat or defatted BSF larvae meal (FBSF or DBSF) mixed with 70% poultry by-product meal (PBM). Furthermore, the effect of supplementing protein source with FBSF could be better. After 8 weeks of feeding, comparing with the control group (0BSF-0PBM), all BSF dietary treatment groups performed no significant difference in the serum immune response, 30FBSF-70PBM group showed no obvious hepatic steatosis, a similar skin mucosal barriers response, and no variation of mortality rate infected with *Vibrio harveyi*. It was unnecessary to defat BSF larvae meal to increase production cost.

Meanhwile Gupta does not recommend complete replacement of FM. The adverse impacts on gut health of fish fed without FM but with 18% BSF and 58% varied non-fishmeal ingredients combination was observed after 7 weeks. In Hender's study, feeding trials of BSF protein (22% BSFP) substituting 30% FM, BSF oil (10.5% BSFO) substituting 30% fish oil (FO), BSF protein and BSF oil (22%/10.5% BSFPO) substituting both 30% FM and 30% FO were respectively conducted for 6 weeks. The results revealed that BSFP diet could increase serum bactericidal activity. Neither BSFP nor BSFO had effect on heat shock proteins related to stress (HSP70 and HSP90). While BSFP and BSFPO significantly upregulated expressions of immune-relevant genes such as interleukin-1β (IL-1β) and interleukin-10 (IL-10), as well as increased the amount of intestinal and skin mucin cells.

4.7 European seabass (*Dicentrarchus labrax*)

Moutinho (2021) found that lipid peroxidation, superoxide dismutase and catalase activities in the liver of European seabass were decreased after 9 weeks at intermediary BSF inclusion levels, while the dietary inclusion of BSF had no effect on activities of glutathione peroxidase and glutathione reductase. He deemed that 19.5% inclusion of BSF can be adopted for the fish, with no affecting on skin colour, liver health, fillet fatty acid profile and quality, meanwhile may decrease liver and fillet lipid oxidation. Abdel-Latif (2021) conducted a similar feeding trial period but with a little lower BSF inclusion level as Moutinho's study. He found the immunostimulatory effects were more obvious, comparing all BSF groups with the control group. In the aspect of antioxidative capacity, malondialdehyde levels and activities of superoxide dismutase, glutathione peroxidase and catalase were all elevated in a dose-dependent manner. In the aspect of non-specific immunity, serum lysozyme content and activities of phagocytic index and respiratory burst were significantly improved; the gene expression of interleukin-1beta (IL-1β), interleukin-10 (IL-10) and heat shock protein 70 (HSP70) in liver were upregulated. In the aspect of disease resistance, the relative survival rate challenged with *Vibrio alginolyticus* was significantly increased. He recommended that up to 50% (inclusion of 15%) dietary FM protein could be replaced by BSF larvae meal for European seabass, with the potential efficacy of antioxidative status, immune responses, and disease resistance, and without adverse impact on fish health.

4.8 Siberian sturgeon (*Acipenser baerii*)

Rawski (2021) and Caimi (2020) respectively research the effects of BSF full-fat and defatted larvae meal for FM replacement on liver and intestine health in Siberian sturgeon. Although the feeding trials were very different, the results were essentially in agreement. In Rawski's study with the inclusion of 10% and 15% full-fat BSF in diets for 7 weeks, the portion of pyloric caeca and proximal intestine were increased, the height and area of villi were enhanced. In Caimi 's study with 18.5% and 37.5% BSF diets for 17 weeks, there was no significant effect on histological index in the liver and distal intestine, but the oxidative stress biomarkers have changed at the inclusion level of 37.5%. In the author's opinion, it doesn't matter whether BSF was defatted or not, the point is to focus on the inclusion proportion of BSF in the feeding formula. To avoid adverse impact on the fish health, high level inclusion of BSF in the diet is not recommended.

4.9 Catfish

A 9-week experiment with BSF larvae substituting FM protein was performed to examine the potential alternative effect of BSF in yellow catfish (*Pelteobagrus fulvidraco*) diet in Xiao's research (2018). Among all tested diets, the highest values of serum lysozyme activity was determined in a diet with 25% substitution (inclusion of 11% BSF). Compared with the control group, increased serum lysozyme activity of fish fed with BSF diets was observed in the maximum replacement of 48% FM (inclusion of 22% BSF). Meanwhile, there were no any negative effects on the index of growth performance and feed conversion rate in this group. So, the author recommended that BSF larvae protein replacing up to 48% of FM protein was feasible for yellow catfish with enhanced the immunocompetence of the fish and without any adverse effects on the growth performance. Hu (2020) conducted a 8-week feeding trial to assess the effects of soybean oil replacement by BSF larvae oil. Although, there was no significant difference in the contents of anti-superoxide anion and malondialdehyde, activities of catalase and superoxide dismutase in the serum of juvenile yellow catfish. Compared with the control group, no significant difference in concentration of tumor necrosis factor-α (TNF-α) was found. When the replacement ratio of BSF oil exceeded 40% (inclusion of 1.6%), the concentrations of anti-inflammatory factor interleukin-10 (IL-10), pro-inflammatory factor interleukin-6 (IL-6) and interleukin-8 (IL-8) in serum of each treatment group were significantly increased, compared with the control group. A 9-week feeding trial was conducted to evaluate the effect of FM replacement with BSF larvae meal on African catfish (*Clarias gariepinus*) fingerlings in Fawole's research (2020). The results are basically like yellow catfish. There was no significant difference in the serum parameters and differential leucocyte counts. The oxidative stress biomarker, malondialdehyde (MDA) content was also similar between BSF based diet and the control, However, the activity of hepatic function enzyme (AST and ALT) was recorded the lowest value in the fish fed with 50% BSF replacement (inclusion of 12%), and the catalase activity improved in this group.

4.10 Zebrafish (*Danio rerio*)

Zebrafish is an important model animal for nutrition research in aquaculture. As an ideal alternative for commercial fish species, it is usually used to preliminarily evaluate the application prospect of novel ingredients before they are applied to aquafeeds (Zarantoniello et al., 2018). There were literatures (Zarantoniello et al., 2018; Lanes et al., 2021) indicated that certain proportion BSF replacing the FM at certain sample time could promote myogenesis-related genes expression. BSF diets could regulate enzymatic hydrolysis activity of chitin. The expression of stress and immune related genes revealed that BSF diets do not impair the fish health. But high BSF substitutions may not play an immunomodulatory role as well as impossible modifications. The above results in zebrafish corresponded to the results in commercial fish species.

4.11 Other species

In the experiment that FM was partially replaced with defatted BSF meal, juvenile clownfish (*Amphiprion ocellaris*, Pomacentridae) did not show significant differences in lipid metabolism and stress response, and intestinal mucosa and liver parenchyma were normal in all experimental groups (Arturo, 2019). Like seabream (*Sparus aurata*), no different indices of poly saturated fatty acids, thrombogenic and peroxidation were observed in the BSF diets groups (Oteri, 2021). Even though the substitution and conclusion proportion in clownfish was high, those in seabream was low, the feeding trial of the two experiments did not overlap at all. After the feeding trial similar with seabream's, immunostimulatory effects were obvious in juvenile large yellow croakers (*Larimichthys crocea*). For example, serum triglyceride (TG) and cholesterol (CHOL) levels significantly decreased with the increase of the replacement level; total antioxidant capacity (T-AOC) of fish liver in the replacing 20% (inclusion of 7%) group and superoxide dismutase activity (SOD) in the replacing 40% (inclusion of 14%) group were the highest among all groups; the lowest liver malondialdehyde (MDA) content was found in the replacing 40% group (Han, 2020). Neither Lu (2020) nor Couto (2022) recommended complete replacement by BSF in fish diets, whatever substituting for FM or soybean meal (SM). Lu found that BSF could improve the catalase activity of grass carp (*Ctenopharyngodon idellus*) when dietary SM complete replacement (inclusion of 26% BSF). However, when the replacement level was higher than 50% (inclusion of 13% BSF), the villi length and wall thickness in intestine decreased, the abundance of *Shewanella* and *Aeromonas* reduced, comparing with the SM group. In Couto's study, eosinophilic granulocytes and intraepithelial leukocytes numbers were increased in juvenile meagre (*Argyrosomus regius*) fed the 50% replacement BSF diet (inclusion of 30%). In addition, plasma anti-protease activity improved with the increase of dietary BSF level. Summary of the effects of substituting BSF in feed ingredients on health of fish are shown in Table 1.

4.12 Conclusion

The application of BSF in fish feed clearly showed that it can replace FM and other ingredients in fish feed. If a small inclusion of BSF is fed to all fish species, whether it is BSF larvae or pupae, stronger immune activity can be seen. It can significantly improve the antioxidant and anti-inflammatory ability of fish, enhance the immune gene expression and disease resistance, and has intestinal antibacterial activity that improves intestinal health. However, high dosage may have side effects on fish health, and the optimal dosage varies with fish species. Therefore, it is suggested to do more studies before the large-scale applications are performed. In a word, BSF is not only a protein alternative feed material, but also can enhance immune activity and improve health levels.

Table 4.1 Effects of Black soldier fly (*Hermetia illucens*) substituting feed ingredients on health of fish

Fish species	Initial weight	Alternative mode	Recommended proportion		Duration	Immunity	Disease resistance	Antioxidant capacity	Anti-inflammation ability	Intestinal flora	References
			Substitution	Inclusion							
Atlantic salmon (*Salmo salar*)	34.00 g	A or B replace α	A: 12.5% B: 6.7%	A: 16.13% B: 35.12%	49 d	(+) skin mucus proteome & immune response		(+) plasma antioxidant capacity	(-) enterocyte steatosis in pyloric caeca, (+) distal intestine histology		Weththasinghe et al., 2021
	1.40 kg	A replace α	100%	15%	112 d					(+) microbial richness & diversity in both digesta- & mucosa-associated intestinal microbiota	Li et al., 2021
		A replace α								(+) relative weight of distal intestine	Li et al., 2020
	49.00 g	A replace β	85%	60%	56 d	(+) genes expression of indicative of stress response & immune tolerance			(+) detoxification activity		Li et al., 2019
Rainbow trout (*Oncorhynchus mykiss*)	201.80 g	A, C or D replace α	45%	30%	35 d					(+) abundance of *Firmicute*, *Actinobacteria*, *Corynebacterium*& *Bacillaceae*; (-) *Proteobacteria*	Huyben et al., 2019

	66.50 g	E replace α	40%	30%	84 d					(+) abundance of *Actinobacteria*, (-) *Proteobacteria*	Terova et al., 2019
	66.50 g	E replace α	50%	30%	84 d					(+) bacterial diversity, (-) *Proteobacteria*, (+) gut abundance of *Mycoplasma*	Rimoldi et al., 2019
	32 g	A and G respectively replace α and γ	100%	16%	70 d	(+) serum lysozyme & peroxidase activities; (+) kidney IL-8, TNF & IRF1			(-) soybean meal induced intestinal enteritis		Kumar et al., 2021
Largemouth bass (*Micropterus salmoides*)	4.90 g	Addition of B		<2%	62 d			(-) genes expression of PPAR-α & lipoprotein lipase, (+) Acetyl-CoA carboxylase 1	Hepatocytes filled with lipids with different degrees of vacuolar degeneration & inflammatory cell infiltration		Peng et al., 2021a
				<18%		(+) immune capacity		(+) antioxidant capacity	(+) intraperitoneal fat deposition		Peng et al., 2021b
	6.02 g	Addition of F		1%	56 d	(+) bactericidal activity	(-) cumulative mortality rates challenged with *Aeromonas hydrophila*	(+) antioxidant activity, (-) oxidative metabolites			Xu et al., 2021
Asian seabass	2.52 g	Mixture containing A replace α	50%	19%	49 d	(+) innate immune responsive					Gupta et al., 2020

(*Lates calcarifer*)						genes expression						
	1.74 g	E or/and G replac α or/and γ	30%	E: 22.1% G: 10.5%	42 d	(+) IL-1β & IL-10; the number of mucin cells in the gut & skin						Hender et al., 2021
	0.56 g	Mixture containing A or D replace α	100%	A: 3.5% D: 2.8%	56 d	No effects in the serum immune response	Normal proportion of infection rate with *Vibrio harveyi*					Chaklader et al., 2021
European seabass (*Dicentrarchus labrax*)	50.00 g	C replace α	45%	19.5%	62 d			Unaffected glutathione peroxidase & glutathione reductase activities				Moutinho et al., 2021
	12.10 g	A replace α	50%	15%	60 d	(+) phagocytic activity, phagocytic index, serum lysozyme & respiratory burst activities; mRNA expression of HSP70), IL-1β & IL-10 genes	(+) the relative percent of survival challenge with *Vibrio alginolyticus*	(+) malondialdehyde levels, & catalase, superoxide dismutase, & glutathione peroxidase enzyme activities				Abdel-Latif et al., 2021

Siberian sturgeon (*Acipenser baerii*)		D replace α	25%	18.5%	118 d			(+) superoxide dismutase activity in mainly kidney, glutathione reductase activity in liver & kidney, catalase activity in both tissues			Caimi et al., 2020
	14.40 g	A replace α and γ	46%	15%	50 d					(+) pyloric caeca & proximal intestine shares, villus height & area	Rawski et al., 2021
Yellow catfish (*Pelteobagrus fulvidraco*)	2.12 g	G replace δ	100%	4%	56 d				(+) IL-10, IL-6, IL-8 & TNF-α		Hu et al., 2020
	48.50 g	A replace α	48%	22.30%	65 d	(+) values of immune indexs					Xiao et al., 2018
African catfish (*Clarias gariepinus*)	4.00 g	E replace α	75%	17.20%	60 d			(+) uperoxide dismutase & catalase enzyme activities			Fawole et al., 2020
Zebrafish (*Danio rerio*)		C replace α	25%	10.5%	7, 14, 21 d post-spawning	(+) HSP70, TNF-α & IL-6, chia.1; (-) nr3c1					Zarantoniello et al., 2018
	5.00 mg	D or C replace α	100%	50%	60 d	(+) chia.2, chia.3, & chia.5 genes expression					Lanes et al., 2021

Clownfish (*Amphiprion ocellaris*)			75%	60%	106 d	Stress response gene expressed normally		Lipid metabolism gene expressed normally	Liver parenchyma was normal	Intestinal mucosa was normal	Arturo et al., 2019
Seabream *(Sparus aurata)*		D replace α	50%	15.7%				No differences were for poly saturated fatty acids, thrombogenic & peroxidation indices			Oteri et al., 2021
Large yellow croaker (*Larimichthys crocea*)	50.08 g		40%	14%	49 d			(+) total antioxidant capacity, (-) liver malondialdehyde content			Han et al., 2020
Grass carp (*Ctenopharyngodon idellus*)	10.10 g	D replace ε	50%	13.4%	56 d			(+) catalase activity		(-) the abundance of *Aeromonas*& *Shewanella*	Lu et al., 2020
Meagre (*Argyrosomus regius*)	18.00 g	E replace α	17%	10%	63 d	(+) numbers of eosinophilic granulocytes & intraepithelial leukocytes					Couto et al., 2022

Note: A denotes full-fat BSF larvae meal; B denotes BSF paste; C denotes BSF prepupae; D denotes defatted BSF larvae meal; E denotes partially defatted BSF larvae meal; F denotes zymolytic BSF pulp; G denotes BSF larvae oil. α denotes fish meal; β denotes protein in the diets; γ denotes fish oil; δ denotes soybean oil; ε denotes soybean meal.

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Chapter 5

LATEST TRENDS IN AQUACULTURE DISEASE MANAGEMENT

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ABSTRACT

Aquaculture is a thriving economy today to address the high demand of fish consumption by humans and to reduce further depletion of wild fish population. But the demand for a booming aquaculture trade has resulted in multiple problems such as destruction of natural habitats, pollution of water sources and particularly, disease outbreaks in both inland and coastal farms. This article briefly discusses the common diseases in farmed shrimps, fish, bivalves and seaweeds with insights on the latest disease mitigation and management methods in aquaculture farms, ranging from traditional methods to advanced molecular techniques.

KEYWORDS: aquaculture, disease, management, shrimp, fish, bivalve, seaweed

5.1 Introduction

Food security is a major issue today especially with the exponential growth of human population and scarcity of crops due to land depletion and exhausted oceans. Seafood such as fish, shrimps and seaweeds are the highest traded food commodity (FAO, 2012; Stentiford et al., 2012; Naylor et al., 2021) and plays a pivotal role in nutritional and financial security in developing countries (Bene et al, 2015). In Malaysia, aquaculture has emerged as the top 15 global producers of seafood with 521,000 tonnes of total aquaculture production (Fathi et al., 2018). The Kyoto Declaration forecast has unfortunately materialised with global aquaculture becoming increasingly important in terms of supplementing capture fishery production (Jennings et al., 2016). The Bangkok Declaration identified management of animal health by cooperative action at national, regional, and inter-regional levels as "an urgent requirement for sustaining growth" (FAO, 2000). Good progress has been made in terms of disease identification, diagnostics, treatment, and zone management but recurring issues, for example lice infestations, are still significant barriers to expansion (Rogers et al., 2016). Infectious diseases caused by viruses, bacteria, and protozoans continue to impose yield-limiting effects on aquaculture production. Additionally, the aquatic environment is a dynamic medium capable of transporting pathogens across vast distances (Munro and Gregory, 2009; Murray, 2013). Pathogens can also be accidentally transferred between farms, due to the movement of organisms and vehicles, creating a convoluted network of pathogen transmission (Munro & Gregory 2009; Murray 2013). To control infectious diseases, large quantities of drugs such as antibiotics have been employed freely resulting in the emergence of resistant organisms (Huang et al. 2015; Lafferty et al. 2015). The most basic way to control diseases prior to medication or technology, would be to manage the environmental parameters (i.e., water quality, culture intensity and temperature), pathogen and aquaculture host. A tilt in the balance or alliance of any of these three factors would offset a disease outbreak. However, despite basic farm practices being followed rigidly, disease outbreaks still occur. This review outlines the different disease management trends for common aquaculture species in Malaysia namely shrimps, fish, bivalves, and seaweeds.

5.2 Disease Management in Shrimp Culture

In the 80's, shrimp aquaculture began with farmers capturing broodstock from the wild introducing these wild broodstocks into natural development cycles (Flegel, 2019). The cultivation of juvenile shrimp from postlarval stage used to occur in earthen ponds with water exchange and the use of pelleted feed, resulting in an increase in the number of ponds as well as in stocking density (Flegel, 2019). The first viral outbreak in shrimps occurred in 1990's, with yellow head virus (YHV) in Thailand and white spot syndrome virus (WSSV) in China and Japan. Figure 5.1 depicts the impact of diseases on shrimp production in Thailand for a duration of about 30 years. The figure showed an increase in shrimp production from 1988 to 1995 when the first occurrence of white spot disease (WSD) began, followed by a period of recovery before another episode in a cyclical manner (Thitamadee et al., 2016; Flegel, 2019). The most serious decline in production was due to early mortality syndrome and acute hepatopancreatic necrosis disease (EMS/AHPND), a devastating disease that wipes out entire populations in tanks within hours. This resulted not only in shrimp mortality, but farmers were reluctant to stock in large amounts until they were sure of successful and healthy harvests (Thitamadee et al., 2016).

Open aquaculture systems are subject to occasional disease outbreaks followed by recovery periods. These diseases are sometimes spread by transboundary movement of infected stocks or by uninfected foreign culture stocks being moved to a new area where they are infected with local pathogens (Flegel, 2006; Flegel & Fegan, 2002).

Twenty viruses have been documented to infect penaeid shrimp (Lightner, 1999). Two types of viruses cause deadly outbreaks: DNA viruses, namely the monodon baculovirus (Lightner & Redman, 1981) and the white-spot syndrome virus (WSSV) (Lo et al., 1996), the hepatopancreatic parvovirus and the infectious hypodermal and haematopoietic virus (Lightner, 1996); and RNA viruses, such as the yellow-head virus (YHV), the Taura syndrome virus (Dhar et al, 2004) and the infectious myonecrosis virus (Poulos et al., 2006). WSSV is the most severe threat for farmed shrimp worldwide and is one of the best-studied crustacean viruses today (Walker, & Mohan, 2009; Sanchez-Pas, 2010; Shekar et al., 2012).

Bacterial infections are a major concern in shrimp aquaculture. *Vibrio harveyi* and *V. vulnificus* cause massive larval mortality (Bachere, 2013; Valente & Wan, 2021), whilst *V. parahaemolyticus, V. damsela*, *V. nichripulchritudo, V. alginolyticus* and *V. penaeicida* cause outbreaks in nurseries or grow-out ponds of shrimps (Bachere, 2013). In 2010, a new disease affecting postlarvae emerged in Asia: a highly virulent strain of *V. parahaemolyticus* that caused the acute hepatopancreatic necrosis disease which acquired a virulent plasmid encoding a pore-forming bacterial toxin (Lee et al., 2015).

The most current methods in mitigating shrimp diseases mostly revolve around DNA technology and biotechnological methods. Farms in Asia are moving towards the production of specific-pathogen free (SPF) stocks that promote faster growth and higher tolerance towards diseases. The introduction of SPF, *Litopenaeus vannamei* in 2000 was so successful that shrimp farmers in Asia only wanted to culture *L. vannamei*. SPF stocks tolerant to TSV were developed using standard breeding and selection methods (Moss et al, 2012). Development of SPF provided farmers with the peace of mind knowing that stocks are ‘clean’ and not going to cause an outbreak.

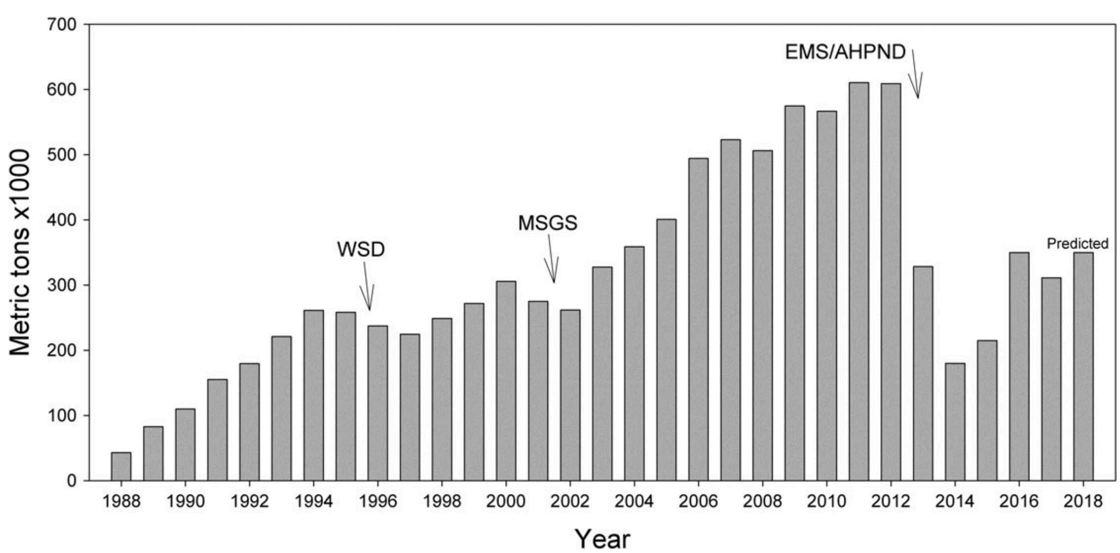

Figure 5.1 Shrimp production in Thailand showing the nature of disease outbreaks. AHPND: acute hepatopancreatic necrosis disease; EMS: early mortality syndrome; MSGS: monodon slow growth syndrome; WSD: white spot disease (adapted from Flegel, 2019).

DNA vaccinations were commonly used but the success of the DNA vaccines are based on the antigen–antibody mechanism and are not applicable to shrimp because shrimps don't produce antibodies (Flegel, 2019). The exact mechanism for vaccination is still being studied as it is not fully understood how injection of viral DNA does protect shrimps particularly from white spot disease (WSD) (Chang et al., 2017).

Shrimps produce a large array of antimicrobial peptides (AMP) against bacteria, viruses, and fungi (Bachere et al, 2000; Tassanakajon et al., 2000). It has been found that certain feed ingredients can regulate semi-specific induction (Flegel, 2019). Some AMPs have broad spectrum antibacterial activity, while some may be more effective against gram-negative bacteria. Because of this, feed manufacturers add specific substances to their feed to boost shrimp's immune response (Destoumieux-Garzon et al., 2016). However, it is possible to design an appropriate molecule that could be added to shrimp feed if the shrimp's immune response system is well understood. This approach allows the binding of selected receptors to up-regulate the AMP, which is perfect for defending against pathogens (Flegel, 2019).

The use of dsRNA to protect shrimp against viruses was designed for WSD (Robalino et al., 2005) and for YHD (Yodmuang et al., 2006). This technology was also successful for other shrimp viruses on which it was tried on (Labreuche & Warr, 2013). Using dsRNA as a feed additive is promising if the production cost can be lowered and if the vaccine can be delivered orally (Itsathitphaisarn et al., 2017).

5.3 Disease Management in Fish Culture

With fisheries playing an enormous role in sustaining food security and livelihood, disease outbreaks in aquaculture are resulting in huge losses of production as well as adding constraints to livelihoods and causing food insecurity. China, a major player in aquaculture production suffered a total fish production loss of 15% to disease outbreaks alone (Leong and Bates, 2014). As the saying "prevention is better than cure" goes, preventing the occurrence of diseases go a long way instead of just treating it (Romera et al., 2012). Improved husbandry practices, vaccine, supplements, probiotics/prebiotics, genetically resistant stock, movement restrictions, non-specific immunostimulants, medicinal plant products, water disinfection, biological control, antimicrobial compounds and disinfection of facility water are the some of the approaches in the control of infectious diseases in fish (Kumar et al., 2016).

Diseases in fish aquaculture are multifold and caused by various pathogens ranging from viruses, bacteria, fungi, protozoans and metazoans. Table 5.1 lists down common pathogenic diseases in fish culture farms.

Table 5.1 Common pathogenic diseases in fish culture farms.

Name of disease	**Causative Pathogen**
Vibriosis	***Vibrio spp. including V. anguillarum, V. harveyi clade, V. parahaemolyticus, Aliivibrio salmonicida (V. salmonicida), V. vulnificus, Photobacterium damselae***
Edwardsiellosis	***Edwardsiella anguillarum, E. ictaluri, E. piscicida, E. tarda, Yersinia ruckeri***
Pseudomonasis	***Pseudomonas anguilliseptica, P. fluorescens***
Aeromonasis	***Aeromonas spp. including Aeromonas caviae, A. hydrophila, A. sobria, A. veronit, A. jandaei, A. salmonicida***
Mycobacteriosis	***Mycobacterium fortuitum, M. marinum, Nocardia asteroides, N. crassostreae (ostreae), N. seriolae***
Streptococcosis	***Streptococcus agalactiae, S. iniae, Lactococcus garviede, Aerococcus viridans***
Channel catfish virus	**Herpesviridae**
Infectious Hematopoietic Necrosis	**Novirhabdoviridae**
Viral Hemorraghic Septicemia	**Novirhabdoviridae**
Viral Erythrocytic Necrosis	**Iridoviridae**
Epizootic Hematopoeitic Necrosis	**Iridoviridae**
Megalocytivirus	**Iridoviridae**
Viral Nervous Necrosis	**Betanodaviridae**
Protozoans	***Ichthyopthirius multifilis*** **(ich)**
	Trichodinia **spp,** ***Trichodonella*** **spp.,**
	Ichthyobodo necator
	Apiosoma **spp.,** ***Ambiphrya*** **spp.**
	Chilodonella **spp.**
	Myxobolus cerebralis **(whirling disease)**
Metazoans	**Isopods**
	Monogeneans
	Copepods

Fish can also suffer from non-communicable diseases resulting from poor environmental conditions and malnutrition. For example, the gas bubble disease that can result in gas-filled lumps on the body and eyes due to excessive dissolved gases in the water. Acidosis is also another environmental disease in fish whereby the pH level of the water is too low, resulting in the water becoming too acidic and fish would suffer from acidosis by swimming rapidly and attempting to jump out of the tank. Nutritional deficiencies in fish can cause the following diseases: scoliosis (as shown in Figure 5.2), lordosis, cataract, anemia, fin erosion, stunted growth, gill hyperplasia and reduced feeding, to name a few.

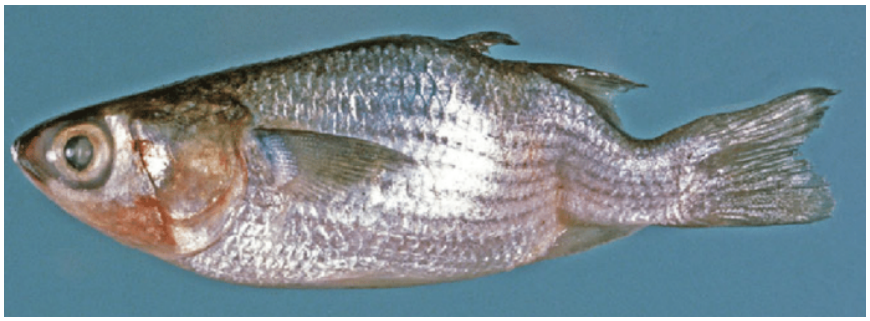

Figure 5.2 Scoliosis and lordosis in a striped mullet, *Mugil cephalus* (adapted from Overstreet & Hawkins, 2017)

A single approach is not enough and cannot sustainably contain a disease as compared to a combination of different strategies which would be more efficient in curbing a disease outbreak in farms. Here, a few approaches to managing diseases along with the most current mitigation methods will be discussed.

Vaccination is an age-old method used to control diseases in aquaculture. Today, there are several variations of vaccination being used in the fish farming scene to manage diseases. Some considerations that should be noted before fish vaccination are the species of fish, production cycle, farming technology, immune status of fish, life history of aquaculture system, the disease in question, the season when the disease occurs, environment, stress factors and costings (Adams, 2016). The types of vaccines used in aquaculture are killed or deactivated vaccines, DNA vaccines, subunit vaccines, genetically-modified vaccines and recombinant vector vaccines (Assefa & Abunna, 2018). Vaccinations are usually done before exposure to a pathogen to allow the fish's immunity to develop. Other factors that determine suitability for vaccination is the size of the fish and temperature of water (Aly & Albutti, 2014). Vaccines can be administered orally, via immersion and via injection.

The use of antibiotics, as prominent as it was back then, is no more a primary treatment option today due to resistance development in fish and an environmental disturbance to the natural microbiota. Due to how disruptive antibiotics can be to both fish and its environment, farmers are advised to use methods apart from antibiotics but if antibiotics must be used due to special circumstances, only approved antibiotics can be administered (Rogers & Basurco, 2009). The American FDA formulated a list of chemicals/antibiotics for aquaculture that has been reviewed (Assefa & Abunna, 2018) including acetic acid, carbon dioxide, hydrogen, sodium chloride, garlic, onion and ice to reduce the metabolic rate of fish during transport (Bentzon-Tilia et al., 2016). If antibiotics are necessary, it is usually administered orally via medicated feed, injection or by immersion.

Probiotics has emerged as a very popular alternative to antibiotics in recent years due to its many advantages, starting from the improvement of internal gut microbiota. The administration of probiotics can be considered a biological disease control because probiotics contain non-pathogenic bacterial strains that multiply within the fish's intestines to normalise microflora and maintain a microbial balance in the fish (Defoirdt et al., 2011). Probiotics are normally administered through diet, encapsulation and immobilized probiotics by direct admission into water. The more commonly used bacterial strains as probiotics in fish farms are *Bacillus subtilis, Lactobacillus acidophilus, Lactobacillus sakei,* and *Shewanella putrefaciens,* and these strains have shown great promise in preventing diseases and promoting weight gain in fish (Assefa & Abunna, 2018).

As opposed to probiotics, prebiotics are also a widely used immune booster and are referred to as "food for probiotics" (Assefa & Abunna, 2018). Some commonly used prebiotics are fructooligosaccharide (FOS), mannan-oligosaccharides (MOS), and mixed oligo-dextran (Carbone & Faggio, 2016).

The use of medicinal plants in aquaculture holds promise as plants have been reported to produce various beneficial effects such as anti-stress, growth promotion, appetite stimulation, immune-stimulation, aphrodisiac and to have anti-pathogenic properties in fish aquaculture due to varied active compounds such as alkaloids, terpenoids, tannins, saponins and flavonoids (Reverter et al., 2017). The active components need to be extracted prior to administration orally, via immersion or injection (Reverter et al., 2017).

Biosecurity measures are also an important factor in managing the spread and outbreak of aquatic diseases in fish farms. Farm-level biosecurity measures involve a combination of activities such as strict quarantine measures, equipment sanitation, disinfection of eggs and facilities, traffic control, water parameter management and treatments, clean feed and appropriate disposal of dead/diseased fish (Assefa & Abunna, 2018). These protocols should be enforced during the introduction of new stocks to avoid transferring pathogens (Assefa & Abunna, 2018).

Improving the genetics of fish is also beneficial in disease management. In Singapore, the Temasik Life Sciences Laboratory (TLL) in collaboration with Marine Aquaculture Centre (MAC) started a breeding programme to improve the Asian sea bass's (*Lates calcarifer*) growth performance, disease resistance and meat quality. TLL developed a library of genomic resources consisting of whole genome sequences, DNA markers, a molecular parentage system, linkage maps, BAC libraries, transcriptomes, and genotyping-by-sequencing platform (Shen et al., 2020). After three generations of within-family selection, the Asian sea bass showed a growth increase of 40% and resistance to the big belly disease, nodavirus and iridovirus (Shen et al., 2020).

5.4 Disease Management in Bivalve Culture

Clam, scallop, oyster and mussel farming represents about 50% of the aquaculture market (Zanella et al., 2017). Bivalve aquaculture can be considered a 'cleaner' form of aquaculture due to a self-regulating cultivation mechanism that requires no additional feed, no antibiotic usage and minimal maintenance effort (Tan, 2021). The farming techniques are simple and can be easily transferred to local growers (Tan, 2015). The oyster was a favorite contender of preferred shellfish in the annual food cycle in Malaysia with the production of oysters coming in third after blood cockles, *Anadara granosa* and green mussels, *Perna viridis* (DOF, 2018; Admodisastro et al., 2021).

The major obstacle in the bivalve aquaculture sector is the emergence and spread of infectious diseases that are intensified by high stocking densities (Guo et al., 2016; Pernet et al., 2016; Zanella et al., 2017). Bivalves are filter feeders and due to this habit, these organisms uptake pathogens while feeding. Some common bacteria found in marine water are those from the genus *Vibrio* and because of how abundantly available these bacteria are, they are often a source for disease outbreaks. Various bacteria species, specifically *Vibrio* (*Vibrio alginolyticus, V. anguillarum, V. tapetis, V. tubiashi, V. neptunius, V. splendidus, V. aestuarianuns* and other *Vibrio* spp.), *Pseudomonas* and *Aeromonas* (Kueh et al., 1985; Prado et al., 2014; Biel et al., 2014) have been reported to cause bacterial diseases of bivalves which are responsible for bacillary necrosis in the larvae of bivalves (Tubiash et al., 1965; Sugumar et al., 1998). The deciliation, velar epithelial loss and abnormal swimming behaviour are caused by exotoxins (ciliostatic factors and hemolysins) produced by *Vibrio* spp. (Valerio et al., 2010).

The most notable bivalve viruses come from the families Herpesviridae and Iridoviridae (Renault and Novoa, 2004; Zanella et al., 2017). These viral pathogens are highly infectious and transmit easily. The increased disease prevalence in bivalve farms is associated to the high-density systems and environmental changes (Guo and Ford, 2016). The detection of a herpes-related viral infection in oyster was first reported by Farley et al. (1972) in *Crassostrea virginica* from the USA. This oyster herpesvirus infection was later shown to be linked with high death rates, especially in larvae and spawn (Segarra et al., 2010). Recent studies also have shown that the oyster, *C. gigas* in Japan, South Korea, Sweden and Norway was infected by OsHV-1 (Bai et al., 2015; Mortensen et al., 2016), causing mass mortality rate. Ultrastructural analysis revealed that larvae displayed generalized infections, while focal infections occurred mainly in juveniles. Adult stages seemed less prone to infections compared to juveniles. A significant reduction in feeding and swimming activities was observed in the infected larvae, resulting in 100% mortality within days. The infection is usually accompanied with the necrosis of the host cell, specifically the epithelium of velar and mantle. Additionally, it also found to be affecting the swimming activity of the larvae. Histological analysis showed that the abnormal cytoplasmic basophilia and enlarged nuclei with marginated chromatin were observed in the fibroblastic-like cells; other cell types including hemocytes and myocytes showed extensive chromatin condensation (Da Silva et al., 2008).

For iridoviruses, “Maladie des branchies” is the first viral disease recorded on bivalves that was responsible for mass mortalities in the Portugese oysters, *Crassostrea angulata*, off the French coasts. This gill disease is caused by gill necrosis virus (GNV) where the gills of infected *C. angulata* usually exhibit intense haemocyte infiltration, abscessing and necrosis. “Maladie des branchies” also affects the Pacific oyster, *C. gigas*, imported in France, but the gill lesions re-occurred with a mild mortality. Due to its natural resistance to infection, *C. gigas* has become the main species bred in Europe.

Another type of Irido-like virus known as the “hemocyte infection virus” was reported as the main cause for the mass mortality of *C. angulata* in France between the years of 1970 and 1973. Histopathological assessment revealed that the discolouration of the digestive gland, disruption in connective tissues and hemocytes infiltration (McGladdery, 2011) was observed in the infected oyster. A third Irido-like virus, the oyster velar virus (OVV), caused high mortalities of larval Pacific oysters on the west coast of North America between the years of 1976 to 1984 (Leibovitz et al., 1998). This virus targeted larvae over 150 μm and usually associated with the lesions in the velum and other ciliated epithelia.

Members belonging to the genera *Haplosporidium, Bonamia, Marteilia* and *Perkinsus* have been identified as the most important protozoan pathogens that target oyster and clam species, causing devastating damage to commercial productions. Diseases caused by *P. marinus, P. olseni, M. refringens, B. ostreae* and *B. exitiosa* are currently under surveillance and require immediate notification by the World Organization for Animal Health. External factors such as temperature and salinity have strong impact on the disease prevalence and intensity of infections (Fernandez Robledo et al., 2014). The pathogen is a lethal infection to the hemocytes, multiplies within blood cells and spreads to all tissues. The pathogen infection is usually accompanied by yellow discoloration in tissues, lesions on gills and mantles, and breakdown of connective tissues. The mortality of bivalves can reach up to more than 90% (Zanella et al., 2017). Apart from the adult, these protozoans can easily infect bivalve larvae. A lack of resistance to infection can easily trigger an epizootic, particularly the high densities of oysters in close-proximity culture beds (Zanella et al., 2017). Perkinosis is a dermo disease caused by *Perkinsus marinus*, a highly contagious protistan protozoan which is responsible for drastic declines in populations of *C. virginica* (Burreson & Calvo, 1996) and *C. gigas, C. rhizophorae* and *C. corteziensis* (Ford, 2011). *Perkinsus olseni* caused perkinosis in clam populations in Australia, Korea, China, Japan and Europe (Park et al., 2010). Transmission of perkinosis occurred directly among the oysters; viable cells were released in host feces or the decomposition of the infected host (Park et al., 2010) and were acquired through host feeding mechanisms. This epidemic disease infects every life stage of bivalves (Paynter et al., 2010) whereby the pathogen infects and proliferates in the digestive epithelium, infects the connective tissue of all organs including hemocytes, causing hemocytosis and tissue lysis with severe emaciation (Smolowitz, 2013). *Perkinsus marinus* causes dermo disease which infects the hemocytes of oysters and subsequently affects their growth and fecundity, eventually leading to death. Another common disease in oysters is MSX, or “multinucleated unknown disease” caused by *Haplosporidium nelsoni* which also results in mass mortalities (Figure 5.3).

Research efforts are focused in exploring inexpensive and effective treatments to prevent production losses when diseases occur in hatcheries (Zanella et al., 2017). The "*OIE Manual of Diagnostic Tests for Aquatic Animals"* has detailed protocols to detect specific pathogens that can be used to confirm histological examination results. With such approaches, it can provide an accurate species-specific diagnosis. Conventionally, the disease-causing pathogens are usually assessed using histopathology; however, the histopathological results today is considered a presumptive diagnosis and requires further confirmatory diagnosis using molecular biology methods such as polymerase chain reaction (PCR), fluorescent *in-situ* hybridization (FISH) and sequencing. The evolution of molecular biology has made PCR-based diagnostic the most valuable method in identifying disease-causing pathogens to prevent and control diseases in bivalve culture. The development of PCR not only permits fast and sensitive screening of pathogens, but it also allows early detection of low infections (Pena, 2002). Apart from PCR, transmission electron microscope (TEM) is also highly recommended to identify and determine the structure of the viruses (Bai et al., 2016).

Marine bivalves are a reservoir of unique bioactive molecules for the development of alternative antimicrobials with AMPs being regarded as encouraging candidates for new and more environmentally friendly antimicrobials. Naturally-derived AMPs are suitable templates for the development of alternatives to antibiotics (Galdiero et al., 2015), and bivalve AMPs are shown to be structurally different from their analogue peptides obtained from the terrestrial habitat (Cheong et al., 2015). One of the most anticipated goals is the use of AMPs with better characteristics, like stronger antibacterial activity, lower cytotoxicity and ease of production for obtaining a marketable drug (Zanella et al., 2017).

Figure 5.3 A healthy oyster (left) versus an oyster infected with *Haplosporidium nelson* (right) (taken from https://barnegatshellfish.org/oyster_disease.htm)

5.5 Disease Management in Seaweed Culture

For centuries, seaweeds are widely consumed in Asia, especially in China (Yang et al., 2017), Japan and Korea (McHugh, 2003). Asian countries ares the major consumers of seaweed products but these products are gaining popularity and are being exported to Europe, North America, and Africa (Chen & Xu, 2005). The popularity and beneficial properties of the seaweeds have triggered the massive expansion of the commercial production of seaweeds. Various species of seaweeds have been studied extensively to understand the potential use of seaweeds as dietary supplements, animal feed, chemicals and biofuels (Tiwari & Troy, 2015). The seaweed aquaculture production is mainly focused within Asia, where it has dominated more than 80% of global production (Kim et al., 2017). Despite the tremendous development of the seaweed aquaculture technologies, this industry still struggles with disease outbreaks, causing the decline in seaweed yields.

Due to the economic importance of *Pyropia*, the best studied seaweed diseases are oomycete pathogens, *Pythium porphyrae* and *Olpidiopsis porphyrae*. Red rot disease caused by *P. porphyrae* was first recorded in Japan (Arasaki, 1947); however, it was only identified as the causative agent after three decades and recognised as the reason of the *Pyropia* crop yield reductions and quality (Takahashi et al., 1977). Initially*, Olpidiopsis* disease was assumed to be caused by a chytrid pathogen, but *Olpidiopsis* spp. was later identified as an obligate endoparasitic oomycete. *Olpidiopsis* disease seems to be endemic as it was only recorded in Japan (Arasaki, 1960; Sekimoto et al., 2008), Korea and China (Kim et al. 2014; Kwak et al., 2017). In Korea, the outbreak of oomycete pathogens is estimated to reduce up to 20% of *Pyropia* yields (Kim et al., 2014), with most of this loss attributed to *O. pyropiae* (Klochkova, et al., 2012) and green-spot disease, caused by the virus PyroV1 (Kim et al., 2016). Despite research efforts on the interactions between oomycete pathogens and their respective *Pyropia h*osts (Klochkova et al., 2012), these studies have offered little insight on biosecurity strategies that can be used in controlling and preventing disease outbreak in culture environments. Recent studies have shown that the red rot disease is not only caused by *P. poryphyrae* but also by another oomycete, *P. chondricola* (Qiu et al., 2019) and the fungus *Alternaria* sp. (Mo et al., 2016). Similarly, *O. pyropia* (Klochkova et al., 2016) has also been found to be the causative agent of *Olpidiopsis* disease, suggesting that the relationship between symptoms and agents may be more convoluted than known.

Ice–ice disease which affects *Kappaphycus* and *Eucheuma* spp., is characterized by thallus whitening in response to environmental stress such as changes in water temperature, salinity and pH, along with the action of opportunistic pathogenic bacteria (Ask & Azanza, 2002). In the past few decades, several bacterial interactions were found to be associated with ice–ice: *Cytophaga-Flavobacterium* complex (Largo et al., 1995) and *Alteromonas - Pseudoalteromonas* (Syafitri et al., 2017). Marine fungi such as *Aspergillus ochraceus*, *A. terreus* and *Phoma* sp. can induce ice–ice symptoms in *K. alvarezii* and *K. striatum* (Solis et al., 2010). Although the development of ice-ice symptoms results from stress to the host from abiotic conditions (Hurtado & Critchley, 2006; Vairappan et al., 2008) together with the action of opportunistic bacteria, the exact mechanisms that instigate the onset and progression of ice-ice symptoms remain unknown to date.

Apart from microorganisms, another significant pest of commercially cultivated seaweeds are epiphytic algae (Ward et al., 2019). Numerous studies have proven that the epiphytic filamentous algae (EFA) are responsible for a reduction in production biomass and carrageenan quality of cultivated *Kappaphycus* and *Eucheuma* spp. in Southeast Asia (Critchley et al., 2004; Hurtado & Critchley, 2006). EFA are found to occur in all major eucheumatoid-producing countries (Pang et al., 2015; Tsiresy et al., 2016). The declines in *K. alvarezii* production in Malaysia are caused by outbreaks of *Polysiphonia* (Hurtado & Critchley, 2006) and die-offs of the same crop species have been attributed to the presence of *Melanothamnus savatieri* in Chinese farms since 2009 (Pang et al., 2011). The fluctuations of abiotic stress such as the seawater temperature, salinity, and current, and intensity of light and nutrient availability not only affect the host strength, but it could trigger the epiphyte infestations (Mtolera et al., 1996; Vairappan, 2006; Pang et al., 2011). (Pang et al., 2011) (Figure 5.4). Epiphytic algae infection causes damage to the cortex of *Kappaphycus* that leads to secondary bacterial infection (Vairappan et al., 2008).

Seaweed diseases have resulted in huge losses as exemplified in Indonesia, the world's largest seaweed producer (Kambey et al., 2020). Over the years, various treatment and mitigation strategies have been introduced to control the impact and spread of disease in the seaweed aquacultures. Acid wash is a commonly used treatment to control all diseases in the seaweed cultivation, particularly *Pyropia* (Kim et al., 2014). Studies have shown that immersion of *Pyropia* blades in acid solutions for more than 5 minutes could prevent the spread of red rot disease (Sakaguchi et al., 2001). This acid wash treatment has found to be partially effective for the red rot disease, cyanobacteria and diatom felt, but it is ineffective for the two most severe diseases of *Pyropia* crops: green-spot disease and *Olpidiopsis* disease (Kim et al., 2014). However, some studies have shown promising results with the use of bioactive metabolites produced by fungal endophytes in inhibiting the infection of *P. yezoensis* by *O. pyropiae* and *P. porphyrae* (Prado et al., 2017; Qiu et al., 2019). Additionally, the change of cultivation conditions with modifications in salinity and light exposure are effective measures in reducing the severity of diseases caused by exposure of *Saccharina japonica* to abiotic conditions (Wang et al., 2014).

Until today, the most effective technique in reducing the impact of pest epiphytes on seaweed production is the constant monitoring of the cultivated populations. This technique allows the farmer to remove those EFA infested crops immediately and replace them with healthy propagules from a different location (Ask & Azanza, 2002). However, there is still no effective management protocols for ice-ice disease to date. Before establishing a cost-effective management protocol, more studies are necessary to understand the underlying mechanisms behind the inset and the progression of ice-ice disease in *Kappaphycus* and *Eucheuma*.

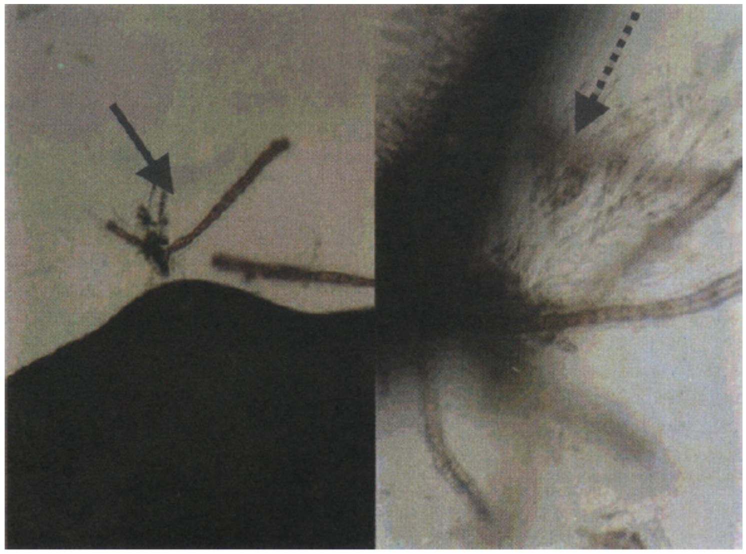

Figure 5.4 A red algal epiphytes (left arrow) *Polysiphonia* and diatoms (right dotted arrow) on *Kappaphycus* thalli (adapted from Largo, 2002)

5.6 Conclusion

To conclude, it is important to remember that an alliance among three factors is necessary for a disease outbreak to happen. This alliance is the “epidemiological triangle” and consists of a host, a pathogen and the environment. It can be concluded that extreme temperatures, varying levels of salinity, human handling, high stocking densities in rearing systems and co-infection with other pathogens may reduce host immunity as well as increase the pathogen’s growth rate and infectivity, resulting in higher susceptibility to a disease. Based on knowledge and research on how a disease can spread whilst acknowledging the role of the environment, improving surveillance on the environment of rearing systems is very important. As knowledge of aquaculture diseases increase, it is important to switch the mindset of one-disease-one-pathogen paradigm to the “pathobiome” concept (Bass et al., 2019). “Pathobiome” systems consist of multiple host-associated organisms associated with decreased host health because of interactions between members of that set, the host, and environmental factors. The paradigm of disease management must shift to management of multiple organisms as well as the use of integrated measures for a healthy and sustainable ecosystem within the aquaculture ecosystem.

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Chapter 6

CURRENT UPDATE ON THE STRATEGIES USED IN COMBATING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

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ABSTRACT

Acute hepatopancreatic necrosis disease (AHPND) is a shrimp disease primarily caused by virulent *Vibrio* species. AHPND has had a devasting directed against the shrimp farming industry with an approximate plunge to 60 % production and has resulted in collective losses of more than USD 43 billion in Asia. This chapter provides an update on the practices being used to mitigate the impact of AHPHD and the ongoing research findings that potentially contribute to new strategies.

KEYWORDS: Acute hepatopancreatic necrosis disease, early mortality syndrome, herbs in aquaculture, probiotic in aquaculture, white leg shrimp.

6.1 Introduction

Vibrio species, that cause acute hepatopancreatic necrosis disease (AHPND), have escalated since the South Asian country collapsed in late 2013. AHPND has a huge negative impact on the shrimp industry and grows rapidly, starting approximately eight days after stocking at postlarvae stage 8 (PL 8), with severe mortality (up to 100 %) occurring within 20 - 30 days (PL 20 - PL 30). As a result, shrimp production in the AHPND-affected areas has temporarily dropped to about 60 % and led to collective losses of more than USD 43 billion across Asia.

Most treatments and control measures developed for AHPND have focused on *Vibrio parahaemolyticus* (VP). However, the presence of a plasmid of AHPND-causing pVA1 with the size of 63–70 kb, encoding toxins PirAVP and PirBVP in non-VP and even non-*Vibrio* species have raised concerns. In addition, the toxin gene seems to migrate horizontally between bacteria (Dong et al., 2017). Hence, the causative agent for AHPND is no longer limited to single species of bacteria. Also, VP cells can replicate in less than fifteen minutes, while other *Vibrio* species take more than an hour.

As the causative agent could be more than one bacteria, investigating management practices to mitigate specific VP is not practical and would put unnecessary economic stress on farmers. Hence, control measures based on the existence of PirABVP toxins in shrimp and aquaculture systems may be more appropriate for the eradication of AHPND in shrimp culture systems. Also, there is interest in investigating methods that can enhance the innate immune responses and enhance disease resistance in shrimp.

In aquaculture, antibiotics is the most common method of treating outbreaks caused by *Vibrio* species; however, this led to the rise of antibiotic resistance, as is the possible spread of drugs in the environment. Consequently, other methods, for instance, disinfection before shrimp post-larvae stocking, pond management, vaccines, supplementation of immunostimulants, prebiotics, probiotics or phages, have gained the interest of farmers in combating AHPND. Nevertheless, there are advantages and disadvantages that farmers need to consider before choosing a method. Therefore, this monograph presents the current strategies and potential methods for treating this disease from the researcher's perspective.

6.2 Current Strategies and Treatments

6.2.1 Antibiotics

VP, which causes early mortality syndrome in white leg shrimp, is a food-borne pathogen caused by bacteria that may cause human gastroenteritis. The bacteria can transmit to humans by consumption of contaminated seafood. Ingestion of VP-contaminated food can cause serious gastrointestinal illness (Tan et al., 2020). To overcome the scenario, antibiotics as feed additives are frequently used in as prevention or treatment (Devi et al., 2009).

The use of antibiotics disrupts microbial maturation systems leading to ineffectiveness in treating diseases caused by *Vibrio* spp. (de Schryver et al., 2014). Anyway, this will lead to another serious threat: multidrug-resistance. In a study by Tan et al. (2020), VP prevalence and antibiotic resistance in blood clams, shrimp, jellyfish and squid were observed. Another research by Lai et al. (2015) concludes that both virulent and non-virulent VP strains are resistant to several antibiotics commonly used in shrimp farming. Nevertheless, inappropriate use of antibiotics in combating AHPND can lead to the development of VP resistant strains. At the same time, *V. campbellii* (that emerged from China), was found to have several genes that encodes with antibiotic resistance (Dong et al., 2017), so traditional methods such as antibiotics were used for shrimp farming. In addition, Dong et al. (2017) revealed that the spread of risk genes in *Vibrio* spp. is likely due to horizontal interspecies gene transfer, allowing VPAHPND to spread between the animals and water, to accelerate plague epidemic disease. Research from (Devi et al., 2009) reveals that a higher total putative VP count and antibiotic resistance were found in pond sediment samples, especially during the pre-monsoon season. Hence, this phenomenon suggests that antibiotic use in shrimp farming should be more strictly regulated.

6.2.2 Disinfection Before Shrimp Postlarvae Stocking

Once AHPND appears in ponds or adjacent ponds, disinfection does not stop the epidemic, so more effective treatments are immediately needed to control AHPND in shrimp. Traditional methods for alleviating or curing VPAHPND strains, for example, alternate feeding or using antibiotics and disinfectants, have had limited benefit (Hong et al., 2016). Thorough bottom of the pond disinfection and water to eliminate possible VPAHPND may help reduce the epidemic outbreak of AHPND disease instead of controling it, and pond management based on microbial control strategies and ecological theory may be essential (de Schryver et al., 2014). Pond management, that involve shrimp culture in microbes' ecosystem balance ecosystems and applying biological control method that in-line with these procedures, are critical in addressing these issues (de Schryver et al., 2014). Furthermore, their use in the food production sector is subject to rigorous research and general scrutiny due to the expansion of multidrug resistance (Cao et al., 2021). For example, VPAHPND from Mexico was reported to carry the tetB gene encoding a tetracycline resistance gene, which shows high levels of resistance to tetracyclines (≥5 g/mL) (Han et al., 2015).

6.2.3 Immune Priming and Quorum Sensing

Immune priming is a memory-like phenomenon described in the invertebrate taxa of animals. For example, using inactivated pathogens against shrimp disease has been reported to induce memory responses in shrimp (Fu et al., 2011). Shrimp-specific immune priming makes "vaccination" desirable and feasible (Fu et al., 2011). Furthermore, it is evolutionarily beneficial for organisms to develop better and faster secondary immune responses to pathogens, which are detrimental and likely to be re-exposed.

Next, invertebrate shrimp are thought incomplete with proper lymphocyte and upper humoral immunity response. Instead, they depend primarily on innate or nonspecific immunity. Immune priming is defined as the two-step vaccination technique in which a pathogen is administrated into the host and revaccinated. For example, upon introducing recombinant viral proteins from White spot syndrome virus (WSSV) into *Bacillus subtilis* spores, immune priming in PLs resulted in huge phagocytic, which is a key biological activity by which the host protects itself from infectious and non-infectious environmental particles and removes unwanted host cells to maintain tissue homeostasis against WSSV (Fu et al., 2011).

Immunity to vibriosis in shrimp vaccinated with pre-formalin-killed *Vibrio* species has a more prolonged postpartum survival (Nguyen et al., 2017). A better immunity to toxin pathogens originated from bacterial, observed in PV post-larvae or larger larvae. Resistance towards the pathogens of antigenic origin initially suggests that the protection elicited by immunization is only for a specific pathogen type and strain in the initial vaccine. Immune specificity is sensitive towards purely harmful bacteria that would harm the host.

On the other hand, antiviral therapy is a promising approach because it only disarms pathogens that affect virulence gene expression instead of killing them. For example, research from de Schryver et al. (2014) reported that element that inhibit quorum sensing and bacterial communication that modulates the expression of virulence factors reduce mortality in giant river shrimp larvae.

6.3 Disadvantages of Current Strategies: Antibiotic Resistance in Shrimp Bacterial Pathogens

The abused widespread use of antibiotics, insecticides and sterilize in aquaculture caused growth of drug-resistant bacteria and caused social concern (Kang et al., 2018) in aquaculture. Research from Kang et al. (2018) isolated and identified 59 VPs (toxR-positive) in shellfish fishing areas on the west coast of Korea between May 2016 and October 2016. The results showed that none of the isolates delimited the tdh and trh virulence genes. The multiple antibiotic resistance mean value of utmost isolates was 0.32, and the highest value was 0.69 in one isolate. Antibiotics are used in aquaculture as feed additives and baths to treatment and prevent the transmit of disease (Rico et al., 2013). Also, VP isolates in some aquatic products from Asian and European countries (e.g., southern China (Xie et al., 2020) have high resistance rates to antibiotics such as ampicillin, rifampicin, and streptomycin (Kang et al., 2018).

6.4 Research Progress and Potential Therapeutic or Control Agents

6.4.1 Probiotics

Probiotics are familiar as prevalent health products for humans, also used in farming. In aquaculture, beneficial bacterial have been broadly used to restrain the evolution of opportunistic pathogens and reduce the prevalence of viruses (Fu et al., 2011). Probiotic usage increased with the need for more environmentally friendly aquaculture practices addition, and probiotics can improve nutrients, enzymatic activity, immune responses, and water quality (Zhou et al., 2009). Using probiotics to inhibit VPAHPND infection or other bacteria in shrimp is practical and improves aquaculture water quality (Kumar et al., 2021). Healthy shrimp can produce digestive enzymes with a healthy gut, and this is often associated with the good expelling of pathogenic bacteria, also in production of elements beneficial for host health (Wang et al., 2020). However, most profit-making available probiotics for aquaculture come from surface-dwelling animals. These non-host are associated with more growing bacteria strains and are relatively void because they cannot stay alive at the intense cell density of the host as well as overcome antagonism during the active growth phase (gut environment) of the shrimp.

6.4.1.1 *Bacillus* spp.

Lim et al. (2020) stated that shrimp intestine microbiota in the wild showed a positive antibacterial effect against VPAHPND. In addition, two isolates showed positive inhibition against VPAHPND, namely *Penicillium* isolate B2 and *Penaeus monodon* isolate BT. In detailed, the isolates B2 and BT are represented as *Bacillus species* based on 16S rDNA sequencing results. The abovementioned bacterial isolates can survive in bile environments, exhibit excellent attachment to the intestinal mucus of shrimp, and are antibiotic-sensitive, non-hemolytic, and non-pathogenic to the host.

Research from Kewcharoen & Srisapoome (2019) isolated probiotic candidates approach coastal areas, and *Bacillus* were tested for the level in mitigating the harmfulness of VPAHPND strains. In addition, *Bacillus subtilis* AQAHBS001, which broadly inhibits various VPAHPND strains, was evaluated on a laboratory scale for water solubility and feed applications of live probiotics. Also, Zhou et al. (2009) showed that the additive of probiotic *Bacillus coagulin* SC8168 taken from shrimp pond sediment in Zhejiang Province, China, could significantly improve PV shrimp's survival rate and partial digestive enzyme (protease, amylase and lipase) activity.

6.4.1.2 Mixed Probiotics

Research from Chumpol et al. (2017) suggested that applying purple non-sulfur bacteria (PNSB) in white shrimp culture would help. Specifically, *Rhodobacter sphaeroides* strains and *Afifella marina* STW181 are beneficial beneficial bacterial for shrimp. However, throughout the research, Chumpol et al. (2017) observed a strong negative correlation between *Vibrio* numbers and shrimp survival. Conversely, a positive correlation was found between PNSB counts and shrimp survival. Another study by Nimrat et al. (2012) examined the efficacy of mixed *Bacillus* probiotic forms and probiotic applications on growth, showing that bacterial counts intersect with water quality LV shrimp culture in separate trials at the larval postlarval stages. The result showed that the form of probiotics and the mode of administration of the probiotics is harmless on the growth and survival of PL shrimp larvae (Nimrat et al., 2012). In addition, *Bacillus* microcapsules and freeze-dried probiotics expressively improved the growth and survival of PL shrimp only. Concerning this, the efficacy of probiotics might depend on exposure time at different stages of individual development (Ziaei-Nejad et al., 2006). Hence, the effectiveness of probiotics will be lesser in the larval period (4 days) into PL stages (22 days).

6.4.2 Phage Therapy

Phage therapies are promising approaches to preventing and treating vibriosis in aquaculture. In addition, phage therapy is a potential management strategy for controlling infectious diseases in humans and animals. The study by Lomelí-Ortega & Martínez-Díaz, (2014) evaluated the efficacy of bacteriophages in preventing and controlling vibriosis in shrimp.

Briefly, VP-infected *Penaeus vannamei* (PV) larvae receive with different phage doses, and the variable used was named phage A3S and Vpms 1. Researchers concluded that one single amount of Vpms1 or A3S phage is sufficient to minimize infection and dying rate in shrimp larvae lead by VP. The outstanding result has overwritten the previous effect from Karunasagar et al., (2019.) and Vinod et al. (2006), where a repeated dose is needed to treat AHPND bacteria phage naturally and is relatively inexpensive, without severe or irreversible side effects reported to date. With this, phage therapy was proven to prevent death by *Vibrio* effectively. In addition, the action of bacterio-phages is very selective, where some can recognize and infect only specific strains, compared to the use of antibiotics. This property is critical to ensure the effectiveness of phages as controls or preventive treatments for the disease in animals (Pereira et al., 2021) because mixed infections are common in cultured organisms. Exciting research from Cao et al. (2021) suggested using multidrug-resistant VP strains as bacteriophages that work as antibacterial agents to control AHPND. Therefore, the study isolated VP phage (vB_VpaP_MGD2) from the clam (*Meretrix meretrix*), targeting multidrug-resistant VP and further characterized it to assess its potential for biocontrol (Cao et al., 2021).

Jun et al. (2016) showed that bacteriophage pVp-1 exhibited significant bacteriolytic activity against three representative VPAHPNDs, resulting in 100% mortality within 24 hours of infection. The mortality of shrimp treated with phage was lower than that of the positive control group. In addition, hepatopancreas of phage-treated shrimp showed a marked region of AHPND with pathological changes after prophylactic treatments. The timing of phage addition concerning pathogen development is critical in vibriosis, which can be addressed by more frequent phage administration.

6.4.3 Plant-Derived Natural Compounds

Herb extracts were studied as an alternative to sustainable aquaculture. The research from Harlina et al. (2022.) focused on the antibacterial potential of *Tribulus terrestris* and basil against *Vibrio harveyi*, *V. alginolyticus* and VP. Research suggested that the organic acids blend (OAB) can significantly increase yield and interspersed to pathogenic *Vibrio*. The action may be a feasible alternative to antibiotics used in the shrimp industry because it exhibits hepatopancreas' protective properties against *Vibrio* spp. (Romano et al., 2015). The OAB includes *Piper betle* and *Cymbo-pogon citratus* extracts incorporated in their normal diet and showed good latent for treating diseases and colds, minimizing AHPND. Research from Immanuel et al. (2004) suggested that certain Ayurvedic herbs compounds have potent effects on the survival of aquatic life and exert specific antibacterial properties. The same studies have shown castor oil, *Phyllanthus niruri*, *Leucus aspera*, *Manihotesculenta* and seaweeds (*Ulva lactuca* and *Sargassum wightii*) can improve protect shrimps from VP infection (Immanuel et al., 2004).

6.4.4 Vaccine

Further research is focused on developing a vaccine against VPAHPND. In general, shrimp will prefer to have passive immunization due to the lack of an adaptive immune system. Specifically, kinetic immunity can protect shrimp from *Vibrio* infection by oral administration of a specific egg yolk powder (IgY) that inhibits VP and *Vibrio harveyi* in vitro (Gao et al., 2016). Relevant research has done just that, demonstrating that specific IgY effectively inhibits the growth of VP, providing passive immunity for shrimp (Hu et al., 2019). Recently, VP declined by the knockout of the LpxDgene gene that caused bacterial virulence and provided substantial protection to shrimp against challenges (Tsai et al., 2019). This method works with attenuated virulent bacteria as live attenuated vaccines against VP. Toxins A aIn addition, since VP can cause AHPND, monoclonal antibodies to toxins A and B that can be used to detect toxins using dot blot (Wangman et al., 2017). Several studies used an anti-Pir A-like toxin IgY diet and demonstrated passive immunity against AHPND (Nakamura et al., 2019). Supplementing anti-Pir A-IgY in feeds be an effective method to prevent AHPND infection. Vaccine development against AHPND is a significant concern as further research is needed to understand the underlying mechanisms and pathogenesis of the disease.

6.5 Conclusion

Shrimp aquaculture is one of the intensified food production sectors in the world. However, the acute hepatopancreatic necrosis disease (AHPND) outbreak has resulted in substantial economic losses to the farming industry since 2009. The causative agent is the virulent *Vibrio* strains, including *V. parahaemolyticus, V. punensis, V. harveyi, V.owensey, V. campbellian and Shewanella* spp. that contains pVA1 plasmids (63–70 kb) encoding the binary PirAVP and PirBVP toxins. AHPND-affected shrimps exhibited notable histopathological changes, including massive shedding of hepatopancreatic epithelial cells without any accompanying signs of pathogens, suggesting that the binary PirAVP and PirBVP toxins secreted by bacteria are involved in the induction of AHPND. Furthermore, recent studies have shown that AHPND-related strains have other specific virulence factors involved in the virulence and disease pathology of AHPND-causing bacteria. Further molecular, microbiological, and histopathological studies are demanded to elucidate the virulence factors of AHPND-causing bacteria. This would help to develop newer strategies for mitigating the disease since most of the methods are still immature to be translated into practical use.

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Chapter 7

INSECT-BASED AQUAFEEDS: A SUSTAINABLE SOLUTION FOR FISH NUTRITION

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ABSTRACT

In routinely used in aquaculture, insect meals have been proven in many studies to be able to replace fish and soya bean meals partially or totally. However, the amino acid and fatty acid composition, as well as the digestibility of various insect diets is insufficient. Hence, research is needed to evaluate the protein, amino acid, lipid, and fatty acid content of insect meal for comprehensive fish nutrition. In general, insect meal contains more essential amino acids than other meal types, with amounts that are like fishmeals, including methionine, which can be a good supply of the essential nutrient. Insects also rich in lipids and fatty acids, and some antimicrobial components like chitin, which is able to inhibit against pathogenic microorganisms, and further enhance animal health. This chapter discusses the beneficial functions of insect based aquafeed for aquaculture

KEYWORDS: Aquafeeds, insect-based feed, marine organisms, protein, feed replacement

7.1 Introduction

The existing global agricultural production system confronts several obstacles in meeting the rising need of animal byproducts, such as livestock, is expected to increase to 58% by 2050 compared to 2010. A revolution within the variety of animal protein production is highly expected to meet the global demand. As a result of the increased need, food resources, particularly the components used in the formulation of feed, may be put under greater strain in aquaculture feed. Conventional ingredients like soybean, wheat, corn, and animal meal are projected to increase in terms of price in the upcoming years (Freccia, *et al*., 2020), this could affect the production cost of aquaculture feed, and the challenges will remain for the next decades. The intensive aquaculture system has been growing in popularity and has emerged as the most promising and practical way to supply the global demand for cultured species. Insect meals offer a novel nutritional source that allows for the highest proportion of fishmeal substitution without compromising the cultivated species' development performance. The fast-growing, immune-enhancing capability and good nutritional quality of insect meals bring probable benefits for cultured species in growth and health promotion (Henry *et al*., 2015).

Fishmeal is processed through by-catch fishery. According to a statistic, global total landings in 2009 were 22.8 million tonnes of non-food fish (25.7%). Of this volume, 17.9 million tonnes (20.2%) of this total were converted into fishmeal and oil meal (FAO, 2012). Fishmeal and fish oil output in the globe has maintained at around 5.0–6.0 million tonnes per year. The soya bean harvest is by far the most widely grown in the world, increasing by 1.4 % from 2010 to 2011. Soya bean output reached 210.9 million tonnes in 2009, representing for slightly more than half of global oilseed meal production (FAO, 2012). Nogales Mérida et al. (2018) found that omnivore and carnivorous fish require higher protein in their diets than the herbivorous or detritivores species. Carnivorous fish require between 55% and 45% crude protein (CP), whereas omnivorous fish require between 45% and 35% CP; these ranges contrast with those of other monogastric vertebrates, which require between 12% and 30% crude protein (CP) (Wilson, 2002). Fish protein needs are directly tied to their amino acid profiles, which are determined by each species' specific amino acid composition.

Fishmeal production output has declined as the marine environment has worsened and fisheries have been depleted, and the price has risen from US$ 600 per metric tonne in 2005 to US$ 2000 per metric tonne in June 2010. During thr year 2010, t he International Monetary Fund (IMF) expects the increased trend in price to continue, with economic implications for animal production. This required for ensuring the need of renewable protein sources, which are especially important in fish diets since fish require a high amount of protein, either from fish feed or perhaps another source (Manzano-Agugliaro *et al*., 2012).

Due to the ecological challenges (deforestation, stock depletion in fishery products, fluctuating cost) caused by the excessive use/over-dependence of fishmeal in aquafeed (SánchezMuros *et al*., 2014, Mousavi *et al.,* 2020). Recurrent environmental concerns, as well as fluctuating fishmeal (FM) production and prices, it has prompted the marine feed sector for an alternate source of protein to meet the growing need for aquafeed in the aquaculture sector. Insect-based aquafeeds, often known as insect meal (IM), have garnered significant international interest as a replacement for FM. Important feeding experiments have shown its potential value not just as a protein replacement, but also as a source of bioactive compounds that boosts immunostimulatory actions in a variety of marine species.

Generally, aquafeeds are made using grains, oilseeds, and marine-origin substances, which may act as an antidote to the current emerging issue. Aquafeeds must also compete for feed components with terrestrial animal industries as pet, chicken, and swine, and complementing ingredients must be developed. Many research have concentrated on alternative sources of protein, but the benefits of plant proteins, microorganism-based proteins, and various animal by-products are currently being addressed some limitations, however significant drawbacks, such as anti - nutritional factors and an uneven nutritional profile, are now being addressed. Throughout this sense, using invertebrates to feed aquatic animals might be another alternative and a critical component of a solution.

The introduction of innovative nutritional sources in commercial aquafeed represents a crucial research field in the sector of aquaculture. Insects have great potential to be nutritious, immune-boost, and sustainable aquafeed that should be tested more in the future. These new sources also offer other advantages as their nutritional value and anti-microbial effect can be modified through intensive culture systems or even with genetic manipulation (Sánchez-Muros *et al*., 2014).

In this case, using insects as a protein supplement for livestock feed is a realistic choice. Insect production on a commercial scale might provide consistent supply in terms of quantity, quality, and price. The use of insect proteins as feed ingredients in aquaculture was permitted by the European Commission in 2017. The revision to the processed animal protein regulations addresses the horizontal slaughterhouse provision for domestic livestocks, which also applies to insect farms and mandates that processed protein be killed in a legally designated slaughterhouse before it can be used in aquafeed. Only 'feed grade' substrates, such as vegetal origin materials or a restricted number of animal origin materials, such as fishmeal, non-ruminant blood products, egg and egg products, milk and milk-based products, honey, and rendered fats, can be given to these insect species (Invertebrate IT, 2021).Some species that are approved by the new EU Regulation 2017/893 entries into force as of 1st July 2017 are as shown in Figure 7.1.

7.2 Benefits of insect meals in terms of physiological improvement and immunity enhancement

Insect meal (IM) has already begun receiving international interest as a potential alternative for FM. Significant feeding studies have demonstrated its potential use not only as a protein substitute but also as a functional element that increases immunostimulatory effects in various aquatic species. In aquatic species, the functional features of IMs, also include their immunomodulatory and physiological effects. Biological metrics such as peroxidation, serum biochemistry, haematological qualities, and immune-related genetics are examples of important factors to examine. In short, low levels of IM supplementation might help specific aquaculture species increase their survival (bivalves, crustaceans etc), immunity, and feed conversion efficiency.

The most important considerations in deciding the choice of IM for aquafeed are nutritional profiles, notably protein and fat levels, nutrient absorption, and insect production. However, the nutritional levels of IM, such as elements (K, Na, Ca, Cu, Fe, Zn, Mn, and P), and vitamin B group vitamins, vitamins A, D, E, K, and C, vary according on sources of food, organisms, and processing techniques. The immunostimulatory effects resulting from the IM are often interpreted in a variety of ways. According to the research, these health-promoting advantages are largely attributed to either direct generation of antimicrobial peptides (AMPs) or indirect transport of chitin or other bioactive chemicals. Furthermore, key molecules in IM, like as AMPs and chitin, might be employed as functional components, allowing the immune-stimulating and antioxidant properties to be fully investigated in order to improve the general health of the aesthetic species. In addition, the immune-stimulating and antioxidant properties of AMPs in insects might make them a natural option to pharmaceutical medications and antibiotic in aquaculture. To understand the functions of AMPs and their consequences on fish physiological and immunological functioning indices in the attempt of natural alternative exploitation, further genetic-based research on screening and extracting AMP genes in IM are needed. To validate the efficacy of IM in illness suppression and management, field studies and in vivo evaluations are required (Mousavi *et al.,* 2020).

Figure 7.1 House cricket (*Acheta domesticus*) (up left); Yellow mealworms(*Tenebrio molitor*) (up right); black soldier larvae (*Hermetia illucens*) (down left); house cricket (*Acheta domesticus*) (down right); Common housefly larvae (*Musca domestica) (Indiviglio, 2022; Indiviglio, 2022; Saanvi, 2021; Heuzé and Tran G, 2015)* and other insects like the lesser mealworm *(Alphitobius diaperinus),* banded cricket *(Gryllodes sigillatus)* and the field cricket *(Gryllus assimilis)*

According to certain studies, chitin stimulates the cell-mediated and systemic immune responses of fish, potentially boosting their resistance to infectious illnesses (Loh, 2017). Immune system response peptides present in IMs, such as AMPs, aid in the protection of the host against bacterial infection. Environmental factors, insect species, nutritional balance in meals, and even the sexes of the insects all influence the kind, amount, and quality of produced AMPs.

Insects have also been shown to have considerable pharmacological capabilities in increasing innate immunity in various species, including humans, animals, and poultry, according to studies. It's also important to understand and study that insects have shown assuring results in treating severe human illnesses such as osteomyelitis, etc.

To study the viability and applicability of FM replacement or IM supplementation in aquafeeds, it is critical to first grasp the physiological and immunomodulatory processes that underpin the concept. The amount of ethereal extract in insect meal varies, and this affects the crude energy (kcal kg^{-1}) of the diets, the energy protein ratio, and the aethereal extract of the carcasses. Insect meals with high levels of aethereal extract were not included as frequently in the diets. High levels of aethereal extract are conducive to fat oxidation (rancidity), which shortens the storage life of this product. It is suggested that antioxidant supplements be used in insect feeds. A short-term option would be to improve pre-processing and production techniques to remove the surplus lipid in the meals and use it as a lipid source in feeds or other industries, similar to how domestic animal by-product meals are made (Arru *et al*., 2019).

Insects may be utilised as a high protein and energy source. Insects also hold a good number of microbial properties. Powdered mealworm has proven to hold microbial properties, while powdered house cricket was characterised by higher microbial diversity since the *Pseudomonas* group was also detected. (Messina *et al*., 2019). Insect larvae have greater aethereal extract levels because they are storing energy for transformation. Its fatty acid composition is very changeable, indicating that when feed is consumed, the insects' fatty acid profile is modulated, maybe as a precursor to the incorporation of lower-quality EPA (eicosapentaenoic acid, $20{:}5n^{-3}$) and DHA (docosahexaenoic acid, $22{:}6n^{-3}$) components. Insect farming helps to natural nutrient recycling and might be a source of high-quality animal protein produced using environmentally friendly technologies.

7.2.1 Chitin as a fibre source

Insect chitin is classified as fibre in general because of its cellulose-like structure. Chitin fibres make up most of an arthropod's exoskeleton (cuticle), and chitin is a polysaccharide made up of glucosamine and N-acetylglucosamine, both of which contain nitrogen atoms (Figure 7.2). Due to the tight coupling of proteins in chitin fibres and variations in chitin according to life stage, estimates of the quantities of chitin and non-digestible protein in insect cuticles vary: hard cuticles have a high protein level of 70–85 percent (dry weight) and a low chitin concentration of 15–30 percent, whereas soft cuticles have a 50 percent chitin and protein content (Chapman 2013; Jonas-Levi & Martinez., 2017).

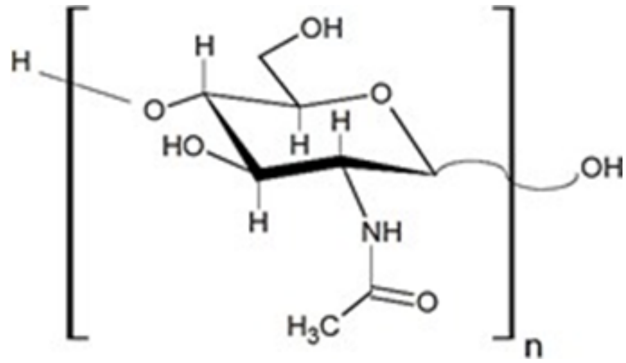

Figure 7.2 Chemical representation of the cellulose like structure of chitin Merzendorfer (2003).

According to Finke (2007), the digestibility of proteins/AAs in insects depends on how much of the AAs are bound to chitin or scleroprotein, which is typically present in adult insect cuticles (Becker & Yu, 2013). Many fish species with 'natural diets,' such as shrimp, crabs, crayfish, and insects, have chitinolytic action from enzymatic reactions like chitinase in the abdomen and chitobiose in the intestinal wall (Lindsay, 1983). These proteins or amino acids can be available for fish nutrition due to chitinolytic activity from enzymes like chitinase in the abdomen and chitobiose in the internal consistency reliability (Gutowska *et al*., 2004).

These chitinase activities were responsible for a reduction in Escherichia coli and caecal Salmonella sp. and an increase in Lactobacillus acidophilus in broilers fed chitin from shrimp meal (Khempaka et al., 2011). Sea bass fed varied levels of chitin (0, 0.5, 1, 2, 3, and 4 g kg^{-1}) showed enhanced growth and feed efficiency up to 2 g kg^{-1} of chitin. Similarly, adding chitin to sea bass fingerlings' meals increased the length of their villus and microvillus in the intestine (Zaki *et al*., 2015). However, as mentioned by Sánchez-Muros *et al. (*2016), the amount of chitin in the diet must be examined since too much chitin might promote weight gain.

7.3 Insect farming for aquafeed

Use of insect meals in animal nutrition demands large-scale insect rearing to provide a steady supply of insects for animal feed. Insect farming is difficult because insects have specific environmental (temperature and humidity), feeding, and population demands, especially during reproduction (Leppla, 2002). Cultivation of insects can be partial (from egg to larva, nymph to adult), entire (from egg to adult), or full (from egg to adult) (egg-egg). The easiest insects to grow are small, multivoltine herbivores that are terrestrial and have simple environmental requirements, such as pest species of crops, stocked items, or gardens (Leppla, 2002). This method of mass raising has become established, particularly for silk production, fishing bait, and pet food (Schabel, 2010).

Recently, Singaporean-based agri-food tech company (Nutrition Technologies Sdn Bhd) has opened their industrial-scale black soldier fly (BSF) farming factory in Malaysia, to develop insect protein. The company has been initiated by building a large network of factories in the region to supply high-quality IM for the aquaculture, pet food, and animal feed industries, which primarily supports circular economy practices throughout the supply chain. More and more companies like ENTOFOOD Sdn Bhd have focused on this insect protein production as an opportunity in establishing a biological process that converts a vast quantity of organic waste into insect biomass and fertiliser every day through the work of detritivorous insects. INSEACT, a Singapore-based company is investing efforts by processing palm oil waste, feeds this to insects and processes the insects into premium ingredients for aquaculture. Life Origin, a social enterprise in Malaysia also focuses on generating positive impact to the environment. They produce environmentally friendly and sustainable protein using food waste and disposable media. These growing number of new start-ups show that the scope for the insect protein as an alternative source would be a major solution towards the increasing demand.

7.4 Constraints and future perspectives on aquafeed

Animal meal and soya bean flour, as well as their derivatives and by-products, are some of the extensively utilised protein sources as feed components. Both are linked to ecological (natural resource mistreatment), economic (feed price fluctuations), or production (quality and quantity variations) issues. Besides the value and dependable commercial-scale manufacturing, one of the key problems with IM inclusion in aqua diets is the nutritional profile range.

Excess fat, amino acid imbalances, mycotoxins, and perhaps antinutritional compounds like chitin are all potential limitations. The immune system and survival rates of animals may be compromised by these individual or cumulative factors. These microscopic organisms, on the other hand, are typically grown in industrial facilities and do not require large amounts of area, particularly when compared to other crops like soybeans. Insects use aids in natural nutrient recycling and provides a high-quality animal protein source made with ecologically friendly methods. The fact that insects are easy to grow and multiply, have a high feed conversion efficiency, and may hunt on organic wastes are all factors in favour of their employment (Mérida *et al*., 2018). Furthermore, research and actual industry demonstrations have proven that supplying big scale insects and using them as a sustainable protein alternative in the diet of marine aqua creatures is technically viable. Once very little animals can be mass produced in large numbers, their costs can be reduced, and they can be used to replace the traditional protein sources in animal feed.

Are insects in aquaculture nutrition a future eco-friendly approach or a commercial reality? It differs according to the industries. As an example, in the salmonid industry, one of the largest and high-value chains in the aquaculture sector, the insect meal already offers an alternative to fishmeal and soya in the early stages of salmon production. The diets were normally made up of the larvae of the black soldier fly (Freccia *et al.,* 2020). These feeds are in use as an EU- approved commodity, and up-to-date surveys show that Norwegian consumers are positive about eating salmon that have had insect meal within the feed formulation. In the future, an academic approach focused on the blue economy will play a significant role in extending consumer acceptance.

7.5 Conclusion

Although insect feed has been examined for use in animal feed, the results have been mixed. Commercial IM production must be produced to match realistic aquaculture market to move an ecologically friendly strategy to the next level. This is also to meet the need for feed meal; enterprises must collaborate with manufacturers that want to enter the commercial market. Furthermore, understanding the nutritional advantages of insects needs research into insect nutrition, since we may be able to change nutrient levels such as EPA and DHA, as well as some essential amino acids, e.g. lysine, methionine, threonine, and tryptophan ratios, based on nutrient requirement of the targeted species. Better understanding is required such as the presence of mycotoxins, lipid rancidification, or unknown antinutritional effects for IM inclusion feed. This field's knowledge is still in its infancy. It is crucial to focus on the separated components from IMs to better understand the process of immunomodulation. the possibilities for using IM in animal nutrition are quite promising, and novel nutritional methods are only being started.

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