Production Efficiency of Asian Seabass (*Lates calcarifer*) Culture Integrated with Sandfish (*Holothuria scabra*) and Tropical Eelgrass (*Enhalus acoroides*)

TONG BENG HUA

THIS DISSERTATION IS SUBMITTED AS PARTIAL FULLFILMENT OF THE REQUIREMENT TO GRADUATE AS BACHELOR OF SCIENCE WITH HONOURS

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ABSTRACT

As aquaculture production increases, integrated aquaculture of organisms from different trophic level is seem to be a way to mitigate the problem of water quality and production efficiency. Hence, an experiment with four treatments was carried out with a land-based recirculating aquaculture system by integrating three components, i.e., Asian seabass (*Lates calcarifer*), Sandfish (*Holothuria scabra*) and Tropical eelgrass (*Enhalus acoroides*). Treatment 'Control' has only Asian seabass, 'With Sandfish' has Asian seabass and Sandfish, 'With Tropical eelgrass' has Asian seabass and Tropical eelgrass and 'All' has Asian seabass, Sandfish and Tropical eelgrass. Treatment 'All' observed the best survival (100%±0.0), weight gained (82.79%±8.84) and feed conversion ratio (1.94±0.06) of Asian seabass. Similarly, Sandfish also recorded higher weight gained in treatment 'All' (102.55%±36.56) compared with treatment 'With Sandfish' (78.21±33.60). This is because Sandfish also excrete ammonia, negatively affecting water quality. Tropical eelgrass in treatment 'All' and 'With Tropical eelgrass' showed inconclusive result as the leave length grown does not reflect the dry weight of the leaves. Although there is no difference in water quality parameters (ammonia, nitrate, nitrite and phosphate), chronic exposure to adverse water quality is most likely to be the cause that results in disease outbreak and thus 100% mortality of Asian seabass in 'Control'. Even though bioremediation of aquaculture waste water can yet to be proven, integrated aquaculture with Asian seabass, Sandfish and Tropical eelgrass is deemed possible and superior to other form of integration.

ABSTRAK

Selaras dengan peningkatan pengeluaran akuakultur, akuakultur bersepadu organisma dari peringkat trofik yang berbeza merupakan salah satu cara untuk mengurangkan masalah kualiti air dan produktiviti. Oleh itu, satu eksperimen telah dijalankan dengan menggunakan empat rawatan dalam sistem integrasi akuakultur berasaskan daratan dengan mengintegrasikan tiga komponen, iaitu, Siakap (*Lates calcarifer*), Putian (*Holothuria scabra*) dan Rumput Laut Tropika (*Enhalus acoroides*). Rawatan 'Control' mempunyai Siakap sahaja, 'With Sandfish' mempunyai Siakap dan Putian, 'With Tropical eelgrass' mempunyai Siakap dan Rumput Laut Tropika dan 'All' mempunyai Siakap, Putian dan Rumput Laut Tropika. Rawatan 'All' menunjukkan kesan yang paling baik dengan kemandiran (100%±0.0), peningkatan berat badan (82.79%±8.84) dan kadar penukaran makanan Siakap (1.94±0.06). Putian juga menunjukkan peningkatan berat badan yang lebih tinggi dalam rawatan 'All' (102.55%±36.56) berbanding dengan rawatan 'With Sandfish' (78.21±33.60). Hal ini kerana Putian juga mengeluarkan ammonia. Rumput Laut Tropika dalam rawatan 'All' dan 'With Tropical eelgrass' (Siakap dan Rumput Laut Tropika) menunjukkan keputusan yang tidak menyakinkan kerana ketumbuhan panjang daun tidak selari dengan peningkatan berat kering daun. Walaupun tiada perbezaan signifikasi dalam parameter kualiti air (ammonia, nitrat, nitrit dan fosfat), pendedahan berpanjangan terhadap kualiti air yang teruk berkemungkinan merupakan punca utama yang mengakibatkan wabak penyakit dan 100% kematian Siakap dalam rawatan 'Control'. Walaupun fungsi bioremediasi sisa air akuakultur tidak dapat dibuktikan, integrase Siakap, Putian dan Rumput Laut Tropika adalah sesuai dan lebih baik.

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LIST OF ABBREVIATION

BFT	= Biofloc Technology
Cm	= centimetre
G	= gram
mg/L	= milligram per litre
ml	= millilitre
mm	= millimetre
pH	= puissance negative de H
RAS	= Recirculating Aquaculture System
SPSS	= Statistical Package for Social Sciences
USD	= United States Dollar

LIST OF SYMBOLS

%	= percentage
°C	= degree Celsius
μm	= micrometer
cm^3	= centimetre

CHAPTER 1

INTRODUCTION

Aquaculture industry, being the fastest growing food-producing industry, has an average annual growth rate of 6.9% (FAO, 2014). Driven by the high demand for seafood, culture system is shifting from extensive polyculture to intensive monoculture (Chopin *et al.*, 2001). To cater to the growing demand, many of the land-based aquaculture operation such as pond culture and hatchery are adopting intensive monoculture. But the sheer volume of aquaculture waste water generated is also diluted in pollutants, hence, any water treatment system has to be very effective in processing the large volume of waste water and removing the pollutants. Because of these impediments, aquaculture waste water is often released into the environment without treatment. This has led to concerns for environmental impacts and sustainability of aquaculture industry. As a result, many attempts including aquaponics, aquasilviculture, biofloc technology (BFT) and integrating aquaculture with agriculture have been developed to address these difficulties (Soto, 2009).

According to Aquaculture Glossary of the Food and Agriculture Organization of the United Nations (Crespi & Coche, 2008), integrated aquaculture is best defined as 'Aquaculture system sharing resources - water, feeds, management, etc. - with other activities; commonly agricultural, agro-industrial, infrastructural (wastewaters, power stations, etc.)'. The aquaculture industry itself can be integrated with different industry such as agriculture while aquaculture species can be integrated with terrestrial plant. Aquaculture species that occupy same trophic level but different niche habitat or with different feeding behaviour such as Phytoplanktophagous surface feeder Silver carp (*Hypophthalmichthys molitrix*) can be integrated with omnivorous bottom feeder Common carp (*Cyprinus* spp.) (Rahman *et al.*, 1992). This form of integration is commonly known as polyculture and it is classified as integrated aquaculture by Troell *et al.* (2009). In addition, integration of aquaculture species from different trophic level is also viable. The working principle of integrating aquaculture species from different trophic level is by utilising the waste products from cultivation system of higher trophic fed aquaculture organism such as fish or shrimp as nutrient source for lower trophic organisms, that is, organic and inorganic extractives (Barrington *et al.*, 2009). Organic extractives such as mussel, oyster and sea cucumber ingest organic waste products (uneaten feed and faecal material) from water while inorganic extractive such as *cottoni* seaweed (*Kappaphycus alvarezii*) removes dissolved inorganic waste (Israel, 2010). The combined effect of organic and inorganic extractives improve water quality and living condition.

According to Soto (2009), many of these attempts were motivated by the advantages integrated aquaculture could bring. By integrating different organisms into a single aquaculture system, water quality could be improved to reduce water exchange rate as evidenced in Biofloc technology (BFT) (Moss *et al.*, 2001) and aquaponics. With improvement in water quality, the optimum environment for aquaculture species would result in better production efficiency such as survival and growth. Integration of different species would eventually diversify the product output of farm and this provides financial security (Chopin *et al.*, 2001). In the case of disease outbreak occurs to one of the species integrated or its market price tumbled, farmers will still be able to generate income from the sale of other organisms. While integrated aquaculture shows potential as the next green technology in aquaculture industry, without scientifically sound data, its application in addressing effluent output of aquaculture industry and improving aquaculture production efficiency are inadmissible.

1.1 Objectives

Hence, the objective of this work is to determine possibility of integrating Asian seabass (*Lates calcarifer*) culture with Sandfish (*Holothuria scabra*) and Tropical eelgrass (*Enhalus acoroides*) by examining its aquaculture production efficiency. Aside from that, this study aims to examine the feasibility of Asian seabass culture integrated with Sandfish and Tropical eelgrass as bioremediation by determining the water quality parameters of this integrated aquaculture system.

1.2 Hypothesis

The hypothesis of this study is that Asian seabass culture integrated with both Sandfish and Tropical eelgrass would yield the best production efficiency and improvement of water quality parameters could also be achieved.

1.2 Significances of Study

This study could bring about significant contributions to aquaculture industry as this integrated aquaculture promotes Asian seabass culture to be integrated with Sandfish and Tropical eelgrass as a viable mean to increase production efficiency of farms without additional capital or input. With the recent increased interest in Sandfish culture, grow out of Sandfish by integrating with existing Asian Seabass culture could also be an economical means of Sandfish mass production. Aside from that, Tropical Eelgrass could be proven as candidate of inorganic extractive for bioremediation of aquaculture waste water. Thus, restoration of Tropical eelgrass bed in natural habitat could be done with transplantation.

CHAPTER 2

LITERATURE REVIEW

2.1 Integrated Aquaculture

Integrated aquaculture has long been practiced in China, Japan and South Korea as traditional means of fish farming, whereby fish is cultured together in an enclosed natural habitat such as bay in vicinity of shellfish and seaweed (Neori *et al.*, 2004) However, optimization of this form of aquaculture was only achieved through trial and error but data was often not published.

In this study, integrated aquaculture of concern is characterized by the integration of two or more components such as fed aquaculture species with organic extractive aquaculture species and inorganic extractive aquaculture species, whereby feed will only be given to the fed aquaculture species. The working principle of this type of integrated aquaculture is that the waste product of fed aquaculture species become the source of nutrient for organic and inorganic extractive. The waste products from the cultivation of fed aquaculture species, usually comprised of faecal material, uneaten feed and other dissolved compounds that would otherwise degrade environmental condition, are assimilated by extractives, producing valuable cash crops (Chopin, 2006).

Hence, a recirculating Asian seabass (*Lates calcarifer*) culture system was integrated with Sandfish (*Holothuria scabra*) and Tropical eelgrass (*Enhalus acoroides*) as part of this experiment. This land-based integrated aquaculture system was a closed system recirculating aquaculture system (RAS) set up in an onshore facility of hatchery in Universiti Malaysia Sabah that allows each treatment to be tested at controlled environment. Water from cultivation of fed aquaculture species were transferred into tanks with organic extractive and then inorganic extractives before being transferred back into fed aquaculture species tank.

2.2 Production Efficiency

Production efficiency in this study generally refers to the cost per unit output of the farm (Bimbao *et al.*, 2000). Hence, survival, growth, weight gained and feed conversion ratio of Asian seabass; survival, growth and weight gained of Sandfish, leaf length grown and dry weight of leaf grown of Tropical eelgrass were measured. With this integrated aquaculture, it is hope that with the same amount of input into the culture system (e.g. feed), the output of integrated aquaculture will be higher than that of other system, as this ecological approach integrate organism of different trophic level to make sure of wastes produced by fed aquaculture species.

2.3 Fed Aquaculture Species

Fed aquaculture species can be fish or shrimp that is the main target species of aquaculture production. The target fed aquaculture species has to be of aquaculture importance and it generally contributes most in income generation of farmers. Feed is usually given only to the fed aquaculture species.

In this experiment, Asian seabass (*Lates calcarifer*) was chosen as fed aquaculture species because this high value species is one of the typical tropical species cultured in Malaysia, highly demanded for its relatively fast growth rate, resilience and high tolerance for turbid water (Chiu *et al.*, 2015).

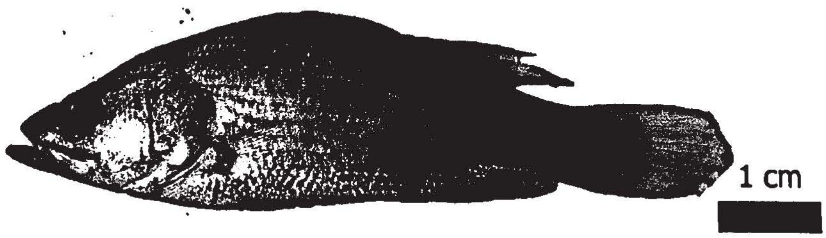

Photo 2.1 Asian seabass as fed aquaculture species.

2.4 Extractives

The defining characteristic of the type of integrated aquaculture of concerns in this study is the cultivation of fed aquaculture species with extractives which can be classified into two categories, namely, organic extractive and inorganic extractive. The candidate of species used as extractive is usually cash crop that can help generate additional income or provide biomitigative services to the culture environment. The combination of organic extractive and inorganic extractive can varies and the possible combination of organic extractive and inorganic extractive is site dependent, species specific, differs with farm design and selection of fed aquaculture species (Chopin, 2006).

2.4.1 Organic Extractive

Organic extractive is responsible to remove organic waste such as fecal material and uneaten feed from fed aquaculture species. They are usually organisms from lower trophic level of fed aquaculture species, such as filter feeder or deposit feeder (Troell *et al.*, 2009). Filter feeder feeds on suspended organic particulates by removing them from water column while deposit feeder ingests organic waste off the surface layer of sediment. For instance, Ferreira *et al.* (2012) has shown that filter feeder Pacific oyster (*Crassostrea gigas*), aside from providing extra revenue-generating products for farmer, it also reduces organic sedimentation to almost 50% of the cultivation of gilthead bream (*Sparus aurata*) in a pond culture setting. Other examples of filter feeder include mussel and oyster (*Chlamys farreri*) (Cheshuk, 2001; Mazzola & Sara, 2001; Langan, 2004) while sea cucumber and sea urchin are the usual deposit feeder used as extractive.

(a) Criteria in Choosing Organic Extractives

Different organic extractive is chosen based on the type of organic waste produced and cultivation method. If organic extractive is cultivated in open water with considerable current and the organic waste produced is neutrally buoyant in water column, filter feeder would be preferred candidate as organic extractive. On the other hand, if the organic waste produced in pond culture is characterized as negatively buoyant particulate, deposit feeder such as sea cucumber is favored.

The type of sediment may also influence the preference over organic extractive of different feeding habits. If the sediment grain size is fine, deposit feeder such as sea cucumber can be used. At site where sediment grain size is coarse, filter feeder can be alternative. However, sediment grain size is usually a function of wave's action. The greater the waves action, the larger the sediment grain size will be, because the energy of waves would carry fine particles away by erosion. Hence, deposit feeder are usually found at area with low current with fine sediment. In contrast, higher current with high turbidity is usually associated and filter feeder. This site specific characteristic become one of the criteria in choosing organic extractive aquaculture species as it require optimal synergies between species mentioned by Cranford *et al.* (2013).

Regardless of feeding habits and sediment grain size, these organic extractive must be able to capture, ingest, assimilate and convert aquaculture waste effectively. Ingestion rate, absorption efficiency, energy budget, and biophysical properties of excreted feces are among the criteria that affect the potential of a species to be the preferred organic extractive in the system. As an example, marine sponge *Chondrilla nucula* Schmidt, 1862 was seem to be a possible candidate of organic extractives for its efficient bacterium *Escherichia coli* retention rate (Milanese *et al.*, 2003).

(b) Sandfish (*Holothuria scabra*) as Organic Extractive

In this experiment, Sandfish (*Holothuria scabra*) was used as organic extractive (**Figure 2.2**). Other species of sea cucumber (*Cucumaria frondosa*) has shown results of great potential in reducing organic loading at cultivation sites (Nelson *et al.*, 2012). Sandfish is a deposit feeder that feed on the surface layer of sediment and exhibit burrowing behavior.

Commonly known as Sandfish with local name of 'Putian', this species of sea cucumber is ranked as one of the species with highest commercial value (Toral-Granda, Lovatelli, & Vasconcellos, 2008). As of February 2012, this species even fetched up to USD 92.50 per kilogram of dry weight (Choo, 2008). In the same paper, Choo also pointed out that their high market demand and commercial value, coupled with their sedentary lifestyle have made them vulnerable to overexploitation to the point where population has depleted by fishing pressure in Malaysia. For the very same reasons, it has driven many attempts in seed production for culture and ranching purposes. Hence, Sandfish is chosen for it may allow adaptation of Sandfish culture into existing fish culture system.

Aside from that, Sandfish was chosen in this experiment because of the ease of its procurement from Pulau Balambangan compared with Green mussel (*Perna viridis*) that was restricted by seasonal spawning. As this experiment employed onshore RAS and that Sandfish was cultivated in sedimentation tank, the wave action or the water current in the sedimentation tank were minimal, hence, deposit feeder Sandfish was chosen to remove organic waste settled at the bottom. In addition, easily available of sand from the beach of Out-Door Development Centre (ODEC) Universiti Malaysia Sabah provide sufficient amount of substrate for this deposit feeder.

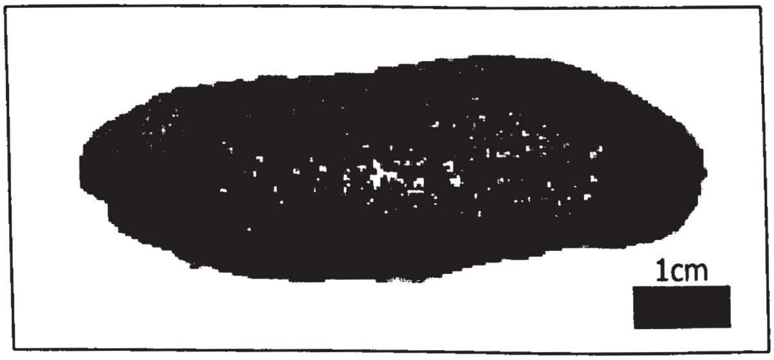

Photo 2.2 Sandfish (*Holothuria scabra*) as organic extractive.

2.4.2 Inorganic Extractive

Inorganic extractives remove inorganic pollutants such as dissolved inorganic nitrogen and dissolved inorganic phosphorus from cultivation system of fed aquaculture species (Chopin *et al.*, 2004). Inorganic extractive aquaculture species is usually autotroph that carry out photosynthesis such as kelp (*Laminaria japonica*, *Saccharina latissima* and

Alaria esculenta) and seaweed (Nunes *et al.*, 2003; Sanderson *et al.*, 2008; Abreu *et al.*, 2009). While kelp (*Laminaria*) has been the prominent candidate for inorganic extractive in temperate fish farming, other seaweed such as *Kappaphycus alvarezii* is often used as inorganic extractive in tropical aquaculture (Soto, 2009). Better nutrient stripping ability of inorganic extractive is preferred.

(a) Criteria of Inorganic Extractive

Inorganic extractives are usually photoautotrophic organisms that absorb dissolved inorganic compounds such as nitrate and phosphate. Its stripping efficiency in removing inorganic nutrient from water is a function of photosynthetic activity, which is dependent of multiples variables including, but not limited to, concentration of carbon dioxide, availability of nutrient and intensity of light.

As kelp is a macroalgae anchored to the substrate of the ocean by its holdfast, the decreasing light intensity with increasing depth can lead to drop in photosynthetic activity. While kelp is naturally adapted to this condition, others inorganic extractives such as '*cottoni*' (*Kappaphycus alvarezii*) have to be cultivated near to water surface for maximum exposure to light.

(b) Tropical Eelgrass (*Enhalus acoroides*) as Inorganic Extractive

In this experiment, Tropical eelgrass (*Enhalus acoroides*) was used as the inorganic extractives. It is a monocotyledon, flowering marine plants with mono-meristematic leaf replacing form growth pattern. Tropical eelgrass can be found in sandy banks from intertidal zone to shallow water of up to 6 meters (Brouns & Heijs, 1986) and its distribution in Indo-Pacific ranges from China, Malaysia to Australian and even to the west, India and Africa. It constitutes the biggest species of seagrass with vegetation of up to two meter height and is characterized with flat, strap-like leaves with rounded, smooth leaf tip (Philips & Menez, 1988). Despite its large size, Tropical eelgrass is slow-growing marine plant that plays successional role of a climax species. For the same reason, it is sensitive to environmental changes as it is not as widely distributed as other fast-growing pioneering seagrass. Despite Tropical eelgrass slow growth, it was chosen as the candidate because it is the largest seagrass species that can form macro habitat and it is also an endangered species.

Unlike kelp or 'cottoni', *Enhalus acoroides* is true plant, angiosperms. They can undergo sexual reproduction when male flower pollinates female flower or asexual vegetative reproduction by rhizome (Hemminga & Duarte, 2000). Their roots anchors them to the sandy substrate near to coastal area and they establish macrohabitat that form vital connectivity between estuarine mangrove areas and coral reef for marine organism to complete their life cycle (Lugendo *et al.*, 2006), even if it is only a small part of their life.

While one may argue that Tropical eelgrass has little or no commercial value now, it is important to know that seagrass bed is one of the critical habitat under-recognized for its environmental services. Threaten by pollution, eutrophication, fouling by epiphytes and coastal development, this plant is suffering from declining population and its role as nursery ground must be acknowledged for it forms macrohabitat vital for marine ecosystem (Short *et al.*, 2011).

Using Tropical eelgrass as inorganic extractive in this experiment could be an effective way of mass cultivating them, thus enabling restoration of Tropical Eelgrass bed by transplantation. This first-in-the-world attempt to cultivate seagrass can have serious implications; if succeeded, this could shed light on the potential of economical mass production of Tropical eelgrass seeds needed for propagation of seagrass bed, restoration of seagrass bed through transplantation or even to replace mangrove plant in aquasilviculture. In addition, as photoautotrophic Tropical eelgrass is carbon-sequester, integrating Tropical eelgrass may contributes to the greening of the tropical aquaculture industry, leading to an industry with negative carbon footprint. Thus, Tropical eelgrass was chosen as the inorganic extractive instead of other species so as to examine the feasibility of it being a candidate of inorganic extractive.

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