REMOVAL OF ORGANIC AND INORGANIC WASTE IN LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE OF SPINY LOBSTER, SEA CUCUMBER AND SEAWEED

MILENNIE VIALANTINE SUMBING

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER IN SCIENCE

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH

2018

UNIVERSITI MALAYSIA SABAH
BORANG PENGESAHAN STATUS TESIS

JUDUL: REMOVAL OF ORGANIC AND INORGANIC WASTE IN LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE OF SPINY LOBSTER, SEA CUCUMBER AND SEAWEED

IJAJAH: IJAZAH SARJANA SAINS (AKUAKULTUR)

Saya MILENNIE VIALANTINE SUMBING, Sesi 2015-2018, mengaku membenarkan tesis Ijazah Sarjana ini disimpan di perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Thesis ini adalah hak milik Universiti Malayisa Sabah
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakann dibenarkan membuat salinan thesis ini sebagai bahan pertukaran antara institusi pengajian tinggi
4. Sila tandakan (/):

[ ]	SULIT	(Mengandungi maklumat yang berdajah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHASIA 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[/]	TIDAK TERHAD	

Disahkan Oleh,

(Tandatangan Pustakawan)

MILENNIE VIALANTINE SUMBING
MY1421029T

Tarikh : 28 Ogos 2018

(Dr. Sujjat Al-Azad)
Pengerusi J/K Penyeliaan

(Prof. Dr. Saleem Mustafa)
Ahli J/K Penyeliaan

(Prof. Madya Dr. Abentin Estim)
Ahli J/K Penyeliaan

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledge.

5 February 2018

MILENNIE VIALANTINE SUMBING

(MY1421029T)

CERTIFICATION

NAME : **MILENNIE VIALANTINE SUMBING**

MATRIC NO. : **MY1421029T**

TITLE : **REMOVAL OF ORGANIC AND INORGANIC WASTE IN LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE OF SPINY LOBSTER, SEA CUCUMBER AND SEAWEED**

DEGREE : **MASTER OF SCIENCE (AQUACULTURE)**

VIVA DATE : **10 JULY 2018**

CERTIFIED BY

Signature

1. CHAIRMAIN

Dr. Sujjat Al-Azad

2. COMMITTEE MEMBER

Assoc. Prof. Dr. Abentin Estim

3. COMMITTEE MEMBER

Prof. Dr. Saleem Mustafa

ACKNOWLEDGMENTS

Firstly, I would like to express my sincere gratitude to my supervisor, Dr. Sujjat Al-Azad for his guidance and assistance in the completion of this dissertation. His helpful supervision, guidance and constant support helped me a lot in completion of this study.

I am also grateful to my Co-supervisors, Dr. Abentin Estim and Professor Dr. Saleem Mustafa for their advice and encouragement. They always welcomed discussion and provided endless motivation throughout the entire research.

I wish to thank the staff of Shrimp Hatchery for their technical assistance and allowing me to use the hatchery facilities. I also would like to thank all the laboratory assistants for their help in laboratory analysis.

I am also thankful to my friends who helped me in collecting the target species for my research. I want to thank all the people that directly or indirectly helped me during this research.

Special thanks to the Ministry of Higher Education Malaysia for providing financial support under the Niche Research Grant Scheme (NRGS0001).

Finally, I would like to thank my parents, Sumbing bin Golot and Sylvia binti Solungki for constant support in every possible way, to all my family members who have always helped me and encouraged me throughout the study.

Milennie Vialantine Sumbing
5 February 2018

ABSTRACT

The rapid development of aquaculture has raised the issues of environmental concern related to the inputs of materials from unsustainable sources and discharge of effluents. Because aquaculture is emerging as an increasingly important sector that can contribute to food security, it has to grow. However, sustainable development of aquaculture requires addressing the issues that undermine its environmental compatibility and value as a source of healthy food choice of consumers. In this context, Integrated Multi-Trophic Aquaculture (IMTA) offers a means of producing aquatic food that has inbuilt systems of mitigating ecological footprint of the farming operations and ensures that the species grown are of high quality. This research was motivated by the needs to demonstrate the relevance of different IMTA designs for production of multiple species that are popular with the consumers and represent practically viable methods that conform to the core principles of ecological aquaculture. The systems approach in the IMTA module stocking of spiny lobster (*Panulirus ornatus*), sea cucumber (*Holothuria scabra*) and seaweed (*Kappaphycus alvarezii*) in land-based facilities is evident in the recirculating system (RS) as well as flow-through system (FTS). A comparison of the two modules reveals their differences in their operational efficiency. The system design consisted of a fibre glass tank (1 m × 1 m × 0.5 m) for spiny lobster and two polyethylene tanks, each measuring 1.3 m × 0.8 m × 0.4 m one which was for sea cucumber and the other one for seaweed. The stocking density were 5 individuals/tank for spiny lobster (mean BW 151.44 ± 7.14 g) and sea cucumber (mean BW 32.16 ± 1.40 g), while the mean initial biomass for seaweed was 500.65 ± 1.76 g/tank. Water flow rate was maintained at 0.08 ± 0.01 L/sec in both the systems. Growth and survival of these species were compared. Water samples from each trophic level were collected every 15 days interval for the analysis of inorganic nutrients. The trial was conducted for 10 weeks. Results indicated that the specific growth rate (SGR) of 0.10 % per day of lobster in RS was not significantly different ($P>0.05$) from its value of 0.13 % per day in FTS. However SGR of seaweed in RS (0.18 % per day) was significantly higher ($p<0.05$) compared to that (0.03 % per day) obtained in FTS. Seaweed removed 50.41%, 48.40%, 31.24% and 31.24% of NH_3-N, NO_2-N, NO_3-N and PO_4-P respectively in FTS. The values of NH_3-N, NO_2-N, NO_3-N and PO_4-P were significantly higher ($p<0.05$) in RS and these parameters appeared to have supported the growth of seaweed. The second experiment was conducted using RS to determine the different diets for spiny lobster and also the performance of seaweed in biofiltration. The stocking rate was 5 individuals/tank for spiny lobster (mean BW 325.61 ± 14.32 g) and sea cucumber (mean BW 56.43 ± 2.33 g), while the initial biomass of seaweed was 504.84 ± 0.59 g/tank. Spiny lobster was fed with three different diets such as, forage fish (Treatment 1), marsh clam (Treatment 2) and commercial shrimp pellet (Treatment 3) for 12 weeks. The feeding frequency was twice daily (8:00h and 16:00h). The result indicated that the SGR of 0.15 ± 0.06 % per day in spiny lobster was significantly higher ($P<0.05$), when fed with dietary treatment 1 compared to SGR (0.12 ± 0.04 % per day) obtained with other two diets. The performance of sea cucumber was lower, with the SGR - 0.06 % per day, - 0.08 % per day and - 0.04 % per day in dietary treatment 1, treatment 2 and treatment 3, respectively. There was no significant difference ($P>0.05$) in the seaweed growth and the dissolved inorganic nutrient (NH_3-N, NO_2-N, NO_3-N and PO_4-P) among the three dietary treatments. The third experiment was conducted in recirculating IMTA system with a similar design with previous experiment. Treatment 1, sea cucumber was given supplementary feed using *Sargassum* powder and treatment 2, no supplementation of *Sargassum* powder in sea cucumber tank. The SGR of sea cucumber was significantly higher (P<0.05), 0.04 % per day in treatment 1 compared to SGR of – 0.05 % per day in treatment 2. The results indicated that the waste generated from spiny lobster tank may not be sufficient for supporting growth of sea cucumber but seaweed efficiently assimilated inorganic nutrients that were generated in the IMTA system. The present study has provided valuable knowledge to the aquaculturist on IMTA system on the potential in reducing the inorganic nutrients and organic matter discharged to the envireonment.

ABSTRAK

PENYINGKIRAN SISA BAHAN-BAHAN ORGANIK DAN BUKAN ORGANIK DI DALAM SISTEM AQUAKULTUR TROPIK BERSEPADU UDANG KARA, MENTIMUN LAUT DAN RUMPAI LAUT

Perkembangan pesat aquakultur telah membangkitkan isu kemampanan alam sekitar yang berkaitan dengan penggunaan bahan daripada sumber yang tidak mampan dan sisa buangan. Akuakultur berkembang sebagai sektor penting yang menyumbang kepada jaminan makanan laut. Oleh itu, pembangunan akuakultur yang mampan diperlukan untuk menangani isu kemampanan alam sekitar sebagai sumber makanan sihat pilihan pengguna. Dalam situasi ini, Sistem Akuakultur Tropik Bersepadu memberikan cara menghasilkan makanan akuatik yang mempunyai sistem pengawalan persekitaran dan memastikan spesis yang ditanam berkualiti tinggi. Penyelidikan ini menunjukan kaitan rekabentuk Sistem Akuakultur Tropik Bersepadu yang berbeza untuk pelbagai spesis sebagai kaedah praktikal yang sesuai dengan prinsip persekitaran akuakultur. Pendekatan Sistem Akuakultur Tropik Bersepadu udang kara (Panulirus ornatus), mentimun laut (Holothuria scabra) dan rumpai laut (Kappaphycus alvarezii) terbukti dalam sistem peredaran tertutup (RS) dan sistem air keluar masuk (FTS). Perbandingan kedua-dua modul ini membuktikan perbezaan kecekapan operasi masing-masing.Sistem ini terdiri daripada tangki gentian kaca bersaiz (1 m × 1 m × 0.5 m) untuk menemptkan udang kara, sebanyak 5 ind/tangki dengan berat purata (BW) 151.44 ± 7.14 g dan dua tangki polietilina bersaiz (1.3 m × 0.8 m × 0.4 m) iaitu masing-masing untuk pengkulturan mentimun laut sebanyak 5 ind/tangki dengan berat purata (BW) 32.16 ± 1.40 g dan rumpai laut dengan jumlah biojisim sebanyak 500.65 ± 1.76 g/tangki. Kadar aliran air untuk kedua-dua sistem tersebut ditetapkan pada 0.08 ± 0.01 L/saat. Sampel air dari setiap tangki diambil setiap 15 hari untuk penentuan kepekatan nutrient inorganik. Percubaan ini dijalankan selama 10 minggu. Hasil kajian menunjukkan tiada perbezaan bererti ($p>0.05$) untuk sistem RS dan FTS iaitu kadar pertumbuhan spesifik (SGR) udang karang ialah 0.10 % per hari dan 0.13 % per hari masing-masing, tetapi sebaliknya bagi SGR rumpai laut, iaitu lebih tinggi ($p<0.05$) di dalam sistem RS iaitu 0.18 % per hari berbanding sistem FTS 0.03 % per hari. Hasil dapatan menunjukkan rumpai laut berupaya menyingkirkan 50.41%, 48.40%, 31.24% dan 31.24% masing-masing kepekatan NH_3-N, NO_2-N, NO_3-N dan PO_4-P dalam FTS. Nilai NH_3-N, NO_2-N, NO_3-N dan PO_4-P lebih tinggi ($p<0.05$) dalam RS dan menyokong pertumbuhan rumpai laut. Experimen 2 dilakukan untuk menentukan diet yang sesuai untuk udang kara dlam sistem RS dan menilai prestasi rumpai laut sebagai bio-penapis. Jumlah stok udang kara dan mentimun laut ialah 5 ind/tangki dengan berat purata masing-masing (325.61 ± 14.32 g) dan (56.43 ± 2.33 g) mnakala biojisim awal rumpai laut ialah 504.84 ± 0.59 g/tangki. Udang kara diberi diet yang berbeza iaitu ikan pemangsa (Rawatan 1), lokan (Rawatan 2) dan pelet udang komersial (Rawatan 3) selama 12 minggu. Pemberian makanan adalah dua kali sehari (8:00h dan 16:00h). Hasil kajian menunjukkan SGR udang kara untuk rawatan 1 ialah 0.15 ± 0.06 % per hari, iaitu lebih tinggi ($p<0.05$) berbanding rawatan 2 dan rawatan 3 iaitu dengan nilai 0.12 ± 0.04 % per hari. Prestasi mentimun laut didapati kurang baik iaitu SGR yang diperolehi ialah -0.06, -0.08 dan -0.04 % per hari masing-masing untuk rawatan 1, rawatan 2 dan rawatan 3 iaitu tiada perbezaan bererti.

Tidak terdapat perbezaan bererti (P> 0.05) untuk pertumbuhan rumpai laut dan nutrien bukan organik (NH_3-N, NO_2-N, NO_3-N dan PO_4-P) di antara ketiga rawatan tersebut. Eksperimen 3 dijalankan menggunakan Sistem Akuakultur Tropik Bersepadu sama seperti reka bentuk sebelumnya untuk mengkaji serbuk Sargassum sebagai makanan tambahan kepada mentimun laut. Rawatan 1, mentimun laut diberi makanan tambahan serbuk Sargassum manakala rawatan 2 tiada serbuk Sargassum diberikan dalam tangki mentimun laut. SGR mentimun laut lebih tinggi (P <0.05), 0.04 % per hari dalam rawatan 1 berbanding dengan SGR -0.05 % per hari dalam rawatan 2. Hasil dapatan ini menunjukan sisa dihasilkan dari tangki udang kara adalah tidak mencukupi untuk menyokong pertumbuhanmentimun laut. Tetapi rumpai laut menunjukan asimilasi nutrien bukan organik dalam sistem Akuakultur Tropik Bersepadu. Kajian ini telah menyumbangkan pengetahuan yang berharga kepada ahli akuakultur pada Sistem Akuakultur Tropik Bersepadu mengenai potensi dalam mengurangkan nutrien bukan organik dan bahan organik yang dilepaskan ke persekitaran.

TABLE OF CONTENTS

CHAPTER 4: RESULTS

LIST OF FIGURES

LIST OF TABLES

LIST OF UNITS

kg m^{-2}	Kilogram per meter square
Kg m^{2} $year^{-1}$	Kilogram meter square per year
km	Kilometer
ha	Hectare
% day^{-1}	Percentage per day
%	Percentage
^{o}C	Degree Celsius
L	Liter
µm	Microliter
mg/L	Milligram per liter
ml	Milliliter
mm	Milimeter
m	Micrometre
cm	Centimeter
g	Gram
g L^{-1}	Gram per liter
g day^{-1}	Gram per day
h	Hour
ppt	Part per thousand

LIST OF ABBREVIATIONS

IMTA	Integrated Multi-trophic Aquaculture
RS	Recirculation system
FTS	Flow-through stsrem
DO	Dissolved oxygen
NH_3-N	Ammonia
PO_4-P	Phosphate
NO_3-N	Nitrite
NO_2-N	Nitrate
BOD	Biochemical oxygen demand
DIN	Dissolved inorganic nutrient
POM	Particulate organic matter
OM	Organic matter
TSS	Total suspended solid
CO_2	Carbon dioxide
PVC	Polyvinyl chloride
HDPE	High density polyethylene
NED	N-(1-naphthyl)-ethylenediamenedihydrochloride
SAN	Sulfanilamide
ODEC	Outdoor Development Center
AOAC	Association of Official Analytical Chemists
H_2SO_4	Sulphuric acid
K_2SO_4	Potassium sulfate
$CuSO_4$	Copper sulfate
HCL	Hydrochloric acid
WG	Weight gain
SGR	Specific growth rate
FCR	Food conversion ratio
CP	Crude protein
MDS	Molt death syndrome

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Aquaculture has become an important economic activity in many counties. Since the global demand for seafood is increasing rapidly, aquaculture has emerged as the fastest growing food production which supplies more than half of the world's seafood (FAO, 2016). On the positive side, expansion of aquaculture has helped us move away from dependence on overexploitation of capture fisheries resources. However, the rapid advancement of aquaculture causes the discharge of significant amount wastewater that leads to environmental problems. The negative environmental impacts of aquaculture industry include pollution of ecosystem with the uneaten feed, excrement waste and metabolite products. Waste products such as inorganic nutrient and organic matter were generated in the monoculture system (Islam, 2005; Herbeck *et al.*, 2013). Effluents from aquaculture activities mainly consist of dissolved inorganic nutrients and particulate organic matter that lead to environmental degradation.

A major challenge faced by aquaculture is minimizing the negative impact on the environment parallel while maximizing the production (Sugiura *et al.*, 2006). Feeds used in farming finfish or shrimp generate the primary waste in the form of faeces and uneaten portion of foods. Species cultured in intensive aquaculture systems generally require large amounts of feed and result in large amounts organic and inorganic waste due to the physiological processes of the different species. An expansion of aquaculture activities has generated severe environmental impact to the environment. Therefore, it is essential to develop system which is environmentally and economically sustainable. Integrated Multi-Trophic Aquaculture

(IMTA) is a current practice by aquaculture farmers which can help to reduce negative aquaculture's impact on the environment.

A way to improve aquaculture sustainability is by reproducing the natural water nutrient cycles in the system. Integrated Multi-Trophic Aquaculture (IMTA) is a new technology which potentially improving the environmental and social sustainability preventing some of the negative ecological impact of aquaculture (Chopin *et al.*, 2001; Troell *et al.*, 2003). IMTA is defined as the farming of fed aquaculture species with the organic and inorganic extractive that inhabit different trophic levels, and enable them to use up the waste products of the fed organisms (Barrington *et al.*, 2009). This involves inorganic extractive seaweed to assimilate dissolved nutrients, filter feeder molluscs to use the suspended organic materials and deposit feeder to consumed settled solids. The incorporation of those species from different trophic levels provide the mutual benefit of the utilization of waste from one species as a nourishment for another species and this benefits the environment (Barrington *et al.*, 2009).

IMTA has been studied and practiced in land-based culture and offshore marine environment. IMTA has currently practiced in over 40 countries that active with aquaculture industry, including United States, Canada, United Kingdom, South Africa, Chile, Israel, China and Japan (Buschmann *et al.*, 2008; Soto, 2009; Chopin, 2011). IMTA concept attempt to mimic the nutrients recycling which involving three trophic levels that allow the nutrients output from fed organisms to serve as feed inputs to the extractive species (Chopin *et al.* 2008). The organic matter such as uneaten feed and faeces from fed aquaculture is consumed by the filter feeders, such as bivalve and mollusc. Sea cucumber which feeds on solids that settle at the sea floor extracts the organic matter. The inorganic nutrients such as nitrogen and phosphorus are used up by seaweed. IMTA probably reduce the net nutrient discharge into the marine environment by recycling the nutrient waste into feed input for another species (Chopin *et al.,* 2007; Ridler *et al.,* 2007). The waste can be recycled as a nutrient for the combined species, and this balance system provides better water quality within the environment.

Integrated aquaculture of fish, abalone, clam and seaweed was established in intensive land-based systems to eliminate the dependence on external food sources, and at the same time reducing the water requirement in the system and nutrient discharge levels (Neori *et al.*, 1998). In sea-based IMTA, more studies have been focused on seaweed for water quality bioremediation (Abreu *et al.*, 2009; Troell *et al.*, 1997). The cultivation of Red algae, *Gracilaria chilensis* in a long line system nearby salmon cage maintained the high growth performance of the seaweed with an average of a 1.7 kg m^{-2} and biomass production of 1500 tonnes from 1 hectare salmon farm (Troell *et al.*, 1997). Abalone (*Haliotis discus hannai*), seaweed (*Ulva lactuca*) and Gilthed seabream (*Sparus aurata*) were successfully used for the land-based integrated culture. The growth rate of *S. aurata* was 0.67 % day^{-1}, *Haliotis discus hannai* obtained 0.9 % day^{-1} and nutrients excreted by the *S. aurata* supported the highest yield of 78 kg m^2 $year^{-1}$ *U. lactuca* which efficiently filterefed 80 % ammonia from the system (Neori *et al.*, 2000). Several IMTA studies have established the efficiency of seaweed as a biolfilters in an integrated aquaculture system (Neori *et al.*, 2000; Huo *et al.*, 2012). Seaweed *G. verrucosa* integrated culture with fish, (*Pseudosciaena crocea*) resulted in the maximum nutrients removal of 60.9 %, 47.7 %, 47.4% and 57.8% for NH_4-N, NO_2-N, NO_3-N and PO_4-P, respectively from the system (Huo *et al.*, 2012).

The species selected in IMTA has the ability to recycle the nutrients thus help to improve the environmental performance of aquaculture sites. Besides their nutrient utilization in the system, the extractive species should be selected for their marketable products intend extra economic benefits to farmers. Selection and arrangement of the species is important so that the lower trophic level could capture both particulate and dissolved waste generated by the higher trophic level. The combination of the species in IMTA system is important according to their complementary roles with another species for their adaptability in relation to habitat and as well as their ability to provide bio-mitigation in the system. Besides that the market demand for the species is also important for commercialization potential (Troell *et al.*, 2009). Hence, the present study was designed to highlight the integrated culture of spiny lobster, sea cucumber and seaweed in land-based systems. These approaches attempt provide better management practices to be applied in aquaculture systems to set the aquaculture sector on the sustainable path.

1.2 Objectives of Study

The main objective of this study was to examine the compatibility and benefits combined culture of spiny lobster (*Panulirus ornatus*), sea cucumber (*Holothuria scabra*) and seaweed (*Kappaphycus* alvarezii) in land-based Integrated Multi-Trophic Aquaculture systems.

The specific objectives of study were:

a) To compare the suitability of spiny lobster, sea cucumber and seaweed in recirculation and flow-through Integrated Multi-Trophic Aquaculture systems.

b) To determine the inorganic nutrients flux in land-based Integrated Multi-Trophic Aquaculture system.

c) To evaluate the effect of different diets provided to spiny lobster, *Panulirus ornatus*, and the waste generated in an Integrated Multi-Trophic Aquaculture system.

d) To determine the performance of seaweed, *Kappaphycus alvarezii*, as a biofilter in the system for inorganic waste from different diets given to spiny lobster.

1.3 Hypothesis

a) Recirculating system would be a suitable candidate for land-based Integrated Multi-Trophic Aquaculture of spiny lobster, sea cucumber and seaweed.

b) The concentration of inorganic nutrients varies in each trophic level within the system.

c) Growth performance of spiny lobster will be better with forage fish but feed wastage will also be much higher when trash fish is used as feed.

d) Seaweeds utilise the inorganic waste and work efficiently as a biofilter in an Integrated Multi-Trophic Aquaculture Systems.

1.4 Significance of Study

a) This study will provide knowledge about suitable modules for Integrated Multi-Trophic Aquaculture of spiny lobster, sea cucumber and seaweed in land-based systems.

b) This information will help in the future development of land-based Integrated Multi-Trophic Aquaculture to scale up towards commercial production.

c) This study also provides the guidelines for aquaculture industry to develop farming systems with sustainable development perspectives.