

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

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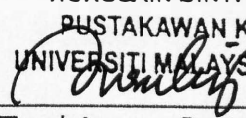
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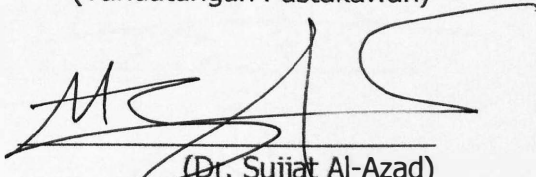
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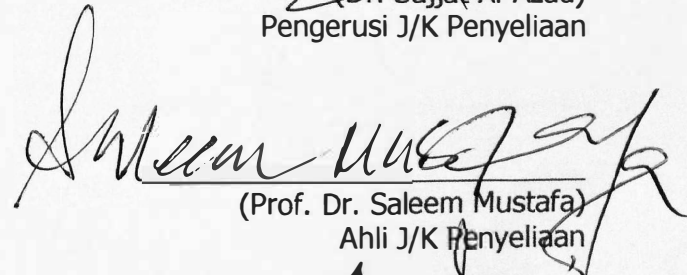
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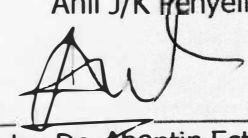

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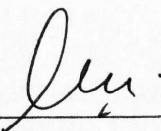

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DECLARATION

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

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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 $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ respectively in FTS. The values of $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ 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 ($\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) 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 environment.



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ABSTRAK

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

Perkembangan pesat akuakultur telah membangkitkan isu kemampunan 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 kemampunan 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 menunjukkan 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 polietilena 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 $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ dan $\text{PO}_4\text{-P}$ dalam FTS. Nilai $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ dan $\text{PO}_4\text{-P}$ lebih tinggi ($p < 0.05$) dalam RS dan menyokong pertumbuhan rumpai laut. Eksperimen 2 dilakukan untuk menentukan diet yang sesuai untuk udang kara dalam 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) manakala 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 ($\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ dan $\text{PO}_4\text{-P}$) 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 menunjukkan sisa dihasilkan dari tangki udang kara adalah tidak mencukupi untuk menyokong pertumbuhan mentimun laut. Tetapi rumpai laut menunjukkan 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.*



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TABLE OF CONTENTS

TITLE	Page i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	v
ABSTRAK	vii
TABLE OF CONTENTS	ix
LIST OF FIGURES	xii
LIST OF TABLES	xv
LIST OF UNITS	xvii
LIST OF ABBREVIATIONS	xviii

CHAPTER 1: GENERAL INTRODUCTION

1.1	Introduction	1
1.2	Objectives of study	4
1.3	Hypothesis	4
1.4	Significance of study	5

CHAPTER 2: LITERATURE REVIEW

2.1	Waste Generated in Aquaculture	6
2.2	Types of Organic and Inorganic Wastes in Aquaculture System	8
2.3	The Integrated Multi-trophic Aquaculture (IMTA)	9
2.4	Role of Integrated Multi-Trophic Aquaculture	10
2.5	Spiny lobster, <i>Panulirus ornatus</i>	11
2.5.1	Feeding Habit of Spiny lobster	12
2.5.2	Water Quality Requirement of Spiny lobster	13
2.6	Sea cucumber, <i>Holothuria scabra</i>	14
2.6.1	Environmental Parameters for Sea cucumber Culture	16
2.6.2	Sea cucumber as organic extractive	17
2.7	Seaweed, <i>Kappaphycus alvarezii</i>	18
2.7.1	Environmental Parameters for Seaweed Growth	19
2.7.2	Temperature (°C)	19
2.7.3	Light intensity (Lux)	20
2.7.4	Seaweed as Biofilter of Dissolved Inorganic Nutrients	21
2.7.5	Seaweed in IMTA System	21

CHAPTER 3: MATERIALS AND METHODS

3.1	Experiment 1: Determination of Suitable System for Integrated Culture of Spiny lobster, Sea cucumber and Seaweed	25
3.1.1	System Design for Land-based IMTA System	25
3.1.2	Spiny lobster Culture Tank	27
3.1.3	Sea cucumber Culture Tank	28
3.1.4	Seaweed Culture Tank	29

3.1.5	Analytical Parameters of Spiny lobster, Sea cucumber and Seaweed	30
3.1.6	Water quality parameters	31
3.1.7	Method for Total Suspended Solids (TSS)	31
3.1.8	Determination of Ammonia (NH ₃ -N)	32
3.1.9	Determination of Nitrite (NO ₂ -N)	32
3.1.10	Determination of Nitrate (NO ₃ -N)	32
3.1.11	Determination of Phosphate (PO ₄ -P)	33
3.2	Experiment 2: Effect of Different Diets Provided to Spiny lobster in the IMTA systems	34
3.2.1	Diet Used in the Feeding Experiment	35
3.2.2	Spiny lobster Cultivation	35
3.2.3	Sea cucumber Cultivation	35
3.2.4	Seaweed Cultivation	36
3.2.5	Analytical Parameters	36
3.2.6	Organic Matter in Sediment	37
3.2.7	Nitrogen in Seaweed Tissue	37
3.2.8	Proximate Analysis	38
3.2.9	Determination of Moisture Content and Dry Matter	38
3.2.10	Determination of Ash Content	39
3.2.11	Determination of Crude Protein	39
3.2.12	Determination of Crude Lipid	40
3.3	Experiment 3: Sea cucumber Feeding Trials	40
3.3.1	<i>Sargassum</i> Powder Preparation	41
3.3.2	Organic Matter Determination in Sea cucumber Sediment	41
3.3.3	Measurement of Sea cucumber	41
3.3.4	Statistical Analysis	41

CHAPTER 4: RESULTS

4.1	Experiment 1: Determination of Suitable System for Integrated Culture of Spiny lobster, Sea cucumber and Seaweed	43
4.1.1	Growth Performance of Spiny lobster (<i>P. ornatus</i>), Sea cucumber (<i>H. scabra</i>) and Seaweed (<i>K. alvarezii</i>) in Recirculation and Flow-through IMTA Systems	43
4.1.2	<i>In-situ</i> Water Quality Parameters during Experiment 1	45
4.1.3	<i>Ex-situ</i> Water Quality Parameter during Experiment 1	47
4.1.4	Nutrient Removal in IMTA module	50
4.1.5	Organic Matter in Sediment and Total Suspended Solid in Water Column	51
4.1.6	Mortality during Experimental Period	52
4.2	Experiment 2: Effect of Diets on the Performance of <i>P. ornatus</i> in the IMTA system	54
4.2.1	Performance of <i>P. ornatus</i> in Experiment 2	54
4.2.2	Proximate Composition of Experimental Diet	55
4.2.3	Carcass Composition of <i>P. ornatus</i>	56
4.2.4	Performance of <i>H. scabra</i> in Experiment 2	57
4.2.5	Organic Matter in the Sediment	58
4.2.6	Performance of <i>K. alvarezii</i> in Experiment 2	59
4.2.7	Total Nitrogen (%) in the Tissue of <i>K. alvarezii</i>	60

4.2.8	Water Quality Parameters during Experiment 2	61
4.2.9	Concentration of Inorganic Nutrients in Different Trophic-level in Experiment 2	62
4.3	Experiment 3: Sea cucumber Feeding Trials	65
4.3.1	Growth Performance of <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> in Treatment 1 and Treatment 2	65
4.3.2	Organic Matter in the Sediment of <i>H. scabra</i> Culture Tank for Treatment 1 and Treatment 2	66
4.3.3	Water Quality Parameters during Experiment 3	67
4.3.4	Inorganic Nutrients Concentration during Experiment 3	68

CHAPTER 5: DISCUSSION

5.1	Experiment 1: Determination of Suitable System for Integrated Culture of Spiny lobster, Sea cucumber and Seaweed	69
5.1.1	Growth Performance of <i>Panulirus ornatus</i> in Recirculating and Flow-through IMTA systems	69
5.1.2	Growth Performance of <i>Holothuria scabra</i> in Recirculating and Flow-through IMTA systems	71
5.1.3	Performance of <i>Kappaphycus alvarezii</i> in Recirculating and Flow-through IMTA systems	72
5.1.4	Dissolved Inorganic Nutrient Removal by <i>Kappaphycus alvarezii</i> in Flow-through System and Recirculation System	74
5.2	Experiment 2: Effect of Diets on the Performance of <i>P. ornatus</i> in the IMTA system	76
5.2.1	Effect of Diets on the Performance of <i>Panulirus ornatus</i> in the IMTA system	76
5.2.2	Performance of <i>Holothuria scabra</i> in Experiment 2	78
5.2.3	Growth performance of <i>Kappaphycus alvarezii</i> in experiment 2	78
5.2.4	Total Nitrogen (%) in the Tissue of <i>Kappaphycus alvarezii</i>	79
5.2.5	In-situ Water Quality Parameters in Experiment 2	80
5.3	Experiment 3: Sea cucumber Feeding Trials	80
5.3.1	Growth Performance of <i>Holothuria scabra</i> with the Supplementation of <i>Sargassum</i> in the Diet	80

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

REFERENCES

82

83

LIST OF FIGURES

	Page
Figure 2.1: Offshore Integrated Multi-Trophic Aquaculture (IMTA) comprises the combination of fed aquaculture (finfish) with organic and inorganic extractive (shellfish and seaweed).	10
Figure 2.2: Spiny lobster, <i>Panulirus ornatus</i>	11
Figure 2.3: Sea cucumber, <i>Holothuria scabra</i>	14
Figure 2.4: Seaweed, <i>Kappaphycus alvarezii</i>	18
Figure 3.1: Design for flow-through system (FTS) with seawater in spiny lobster tank and outlet at seaweed tank. Original setup (a), Schematic diagram (b)	26
Figure 3.2: Design for recirculation system (RS) with water reuse. Original set up (a), Schematic diagram (b)	26
Figure 3.3: Spiny lobster, <i>P. ornatus</i> , stocked at the rate of five individuals/500 L fibreglass tank	27
Figure 3.4: Sea cucumber, <i>H. scabra</i> stocked at the rate of five individuals/500 L polyethylene tank (top view)	28
Figure 3.5: Seaweed, <i>K. alvarezii</i> , attached to a tie-tie rope in 500 L polyethylene tank	29
Figure 3.6: A schematic diagram of recirculation system in the Integrated Multi-Trophic Aquaculture used for spiny lobster, seaweed and sea cucumber tank	34
Figure 3.7: Experimental layout for three different dietary treatments for spiny lobster (triplicate set)	34
Figure 3.8: Experimental diets for the spiny lobster	35
Figure 3.9: Seaweed, <i>K. alvarezii</i> culture in a net cage in a 500 L polyethylene tank	36
Figure 3.10: <i>Sargassum</i> sample collected from Pulau Sepanggar (a) and <i>Sargassum</i> powder ready for use in feeding sea cucumber (b)	41
Figure 4.1: Trend in the concentration of dissolved inorganic nutrient $\text{NH}_3\text{-N}$ in recirculation and flow-through system during a 10 week experimental period	48

Figure 4.2:	Trend in the concentration of dissolved inorganic nutrient NO ₂ -N in recirculation and flow-through system during a 10 week experimental period	48
Figure 4.3:	Trend in the concentration of dissolved inorganic nutrient NO ₃ -N in recirculation and flow-through system during a 10 week experimental period	49
Figure 4.4:	Trend in the concentration of dissolved inorganic nutrient PO ₄ -P in recirculation and flow-through system during a 10 week experimental period	49
Figure 4.5:	Contents of organic matter (%) in sediment from <i>H. scabra</i> culture tank (mean \pm SD) during the 10 week experimental trial	51
Figure 4.6:	Mean \pm SD of total suspended solids (mg/L) determined in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks designed for RS and FTS	52
Figure 4.7:	Evidence of cannibalism in the <i>P. ornatus</i> during the trials	53
Figure 4.8:	Live Pea crab observed emerging out from the dead <i>H. scabra</i> at weeks 6 and 7	53
Figure 4.9:	(a) <i>K. alvarezii</i> was infected by ice-ice disease in first week and (b) <i>K. alvarezii</i> started to grow in the second week with the formation of new thallus	53
Figure 4.10:	Organic matter (%) in the sediment of <i>H. scabra</i> given different diets to the <i>P. ornatus</i>	58
Figure 4.11:	<i>K. alvarezii</i> in the recirculation IMTA module at first week of the experiment	60
Figure 4.12:	<i>K. alvarezii</i> in the recirculation IMTA module after 12 weeks of the experiment	60
Figure 4.13:	Concentration (mean \pm SD) of (a) Ammonia (mg/L) in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks with three different diets (N=18)	63
Figure 4.14:	Concentration (mean \pm SD) of Nitrite (mg/L) in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks with three different diets (N=18)	63
Figure 4.15:	Concentration (mean \pm SD) of Nitrate (mg/L) in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks with three different diets (N=18)	64

Figure 4.16: Concentration (mean \pm SD) of Phosphate (mg/L) in *P. ornatus*, *H. scabra* and *K. alvarezii* tanks with three different diets (N=18) 64

Figure 4.17: Percentage of organic matter (mean \pm SD) in the sediment of *H. scabra* tank in treatment 1 (fed with *Sargassum*) and treatment 2 (without *Sargassum*) during the 10 week feeding trial 66



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UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

	Page
Table 2.1: Tolerance suitability of temperature (°C) based on <i>Euchema</i> sp. and <i>Kappaphycus</i> sp.	20
Table 2.2: Maximal removal efficiency of nutrients by some seaweeds in integrated cultivation with several marine animals.	22
Table 4.1: Weight gain, specific growth rate and survival of spiny lobster (<i>P. ornatus</i>), sea cucumber (<i>H. scabra</i>) and seaweed (<i>K. alvarezii</i>) in recirculation system (RS) and flow-through system (FTS)	44
Table 4.2: Mean (\pm SD) and range of <i>in situ</i> water quality parameters recorded in the recirculation system (RS) and flow-through system (FTS) during 10 weeks of the experiment	46
Table 4.3: Dissolved inorganic nutrients removal by <i>K. alvarezii</i> in RS and FTS	50
Table 4.4: Growth performance (mean \pm SE) of <i>P. ornatus</i> fed three different diets during 12-weeks of culture period	54
Table 4.5: Proximate composition of experimental diets on dry wet basis	55
Table 4.6: Carcass composition of <i>P. ornatus</i> flesh fed with different diets in experiment 2 (dry weight basis)	56
Table 4.7: Performance (mean \pm SE) of <i>H. scabra</i> in the 12 week culture period	57
Table 4.8: Growth performance of <i>K. alvarezii</i> in IMTA module with spiny lobster fed with three diets in 12-weeks of the experimental trial	59
Table 4.9: Percentage of Total Nitrogen in <i>K. alvarezii</i>	61
Table 4.10: <i>In situ</i> water quality parameters recorded in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks during the 12-week experiment	62
Table 4.11: Growth performance of <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> in Treatment 1 and Treatment 2	65

Table 4.12	In situ water quality parameters recorded in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> tanks during the 10-week experiment	67
Table 4.13	Concentrations (mean \pm SD) of Ammonia (mg/L), Nitrite (mg/L), Nitrate (mg/L) and Phosphate (mg/L) in <i>P. ornatus</i> , <i>H. scabra</i> and <i>K. alvarezii</i> in Treatment 1 (T1) and Treatment 2 (T2).	68



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UNIVERSITI MALAYSIA SABAH

LIST OF UNITS

kg m⁻²	Kilogram per meter square
Kg m² year⁻¹	Kilogram meter square per year
km	Kilometer
ha	Hectare
% day⁻¹	Percentage per day
%	Percentage
°C	Degree Celsius
L	Liter
μm	Microliter
mg/L	Milligram per liter
ml	Milliliter
mm	Millimeter
m	Micrometre
cm	Centimeter
g	Gram
g L⁻¹	Gram per liter
g day⁻¹	Gram per day
h	Hour
ppt	Part per thousand



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LIST OF ABBREVIATIONS

IMTA	Integrated Multi-trophic Aquaculture
RS	Recirculation system
FTS	Flow-through system
DO	Dissolved oxygen
NH₃-N	Ammonia
PO₄-P	Phosphate
NO₃-N	Nitrite
NO₂-N	Nitrate
BOD	Biochemical oxygen demand
DIN	Dissolved inorganic nutrient
POM	Particulate organic matter
OM	Organic matter
TSS	Total suspended solid
CO₂	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
H₂SO₄	Sulphuric acid
K₂SO₄	Potassium sulfate
CuSO₄	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 countries. 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 Gilthead 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 m² year⁻¹ *U. lactuca* which efficiently filtered 80 % ammonia from the system (Neori *et al.*, 2000). Several IMTA studies have established the efficiency of seaweed as a biofilters 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 NH₄-N, NO₂-N, NO₃-N and PO₄-P, respectively from the system (Huo *et al.*, 2012).

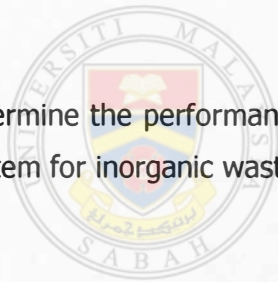
The species selected in IMTA has the ability to recycle the nutrients thus help to improve the environmental performance of aquaculture sites. Besides their nutrient utilization in the system, the extractive species should be selected for their marketable products intend extra economic benefits to farmers. 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.



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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.



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