GROWTH AND NUTRITIONAL CONDITION OF SEA CUCUMBER (HOLOTHURIA SCABRA) IN CULTURE SYSTEMS

SOFIA JOHARI



BORNEO MARINE RESEARCH INSTITUTE UNIVERSITI MALAYSIA SABAH 2007

KONDISI TUMBESARAN DAN NUTRISI TIMUN LAUT (HOLOTHURIA SCABRA) DALAM SISTEM KULTUR

SOFIA JOHARI

PERPUSTAKALAN UNIVERSITI MALAYSIA SABA''

TESIS INI DIKEMUKAKAN UNTUK MEMENUHI SYARAT MEMPEROLEHI IJAZAH SARJANA SAINS

INSTITUT PENYELIDIKAN MARIN BORNEO UNIVERSITI MALAYSIA SABAH 2007

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS[®]

JUDUL : GROWTH AND NUTRITIONAL CONDITION OF SEA CUCUMBER (HOLOTHURIA SCABRA) IN CULTURE SYSTEMS

IJAZAH : SARJANA SAINS (AKUAKULTUR)

SESI PENGAJIAN : 2003-2007

Saya, SOFIA BINTI JOHARI mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

- 1. Tesis adalah hakmilik Universiti Malaysia Sabah
- 2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian saya
- 3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi
- 4. TIDAK TERHAD

Disahkan oleh

ANITA BINTI ARSAD

(Penulis : SOFIA BINTI JOHARI)

Alamat:

Suleun MUS

(Penyelia : Prof. Dr. Saleem Musiafa)

(TANDATANGAN PUSTAKAWAN)

Tarikh : 28 Mac 2007

Tarikh: 07.05.07

CATATAN : [@] Tesis dimaksudkan sebagai tesis Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau laporan Projek Sarjana Muda (LPSM)

DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

SOFIA JOHARI PS 03-004-001 28 MARCH 2007



ACKNOWLEDGEMENTS

I would like to thank a lot of people for helping me during my thesis preparation period. First and foremost, my heartiest gratitude goes to my principal supervisor, Prof. Dr. Saleem Mustafa for his guidance, support and patience. My gratitude also goes to Miss Annita Yong as my co-supervisor.

I thank the Ministry of Science, Technology and Environment, Malaysia for providing financial support through grants IRPA 01-02-10-0014 and IRPA 01-02-10-0018. I would also like to acknowledge Tun Fuad Foundation for providing scholarship during my study.

My thank also goes to Prof. Ridzwan Abdul Rahman and Dr. Mabel Manjaji Matsumoto for giving me the permission to share and use the wet laboratory. Special thanks to Gayana Resort for giving me a place to do several experiments at their Marine Research Centre, especially to Mr. Zamrie and Mr. Asri.

I would also like to express my appreciation to Mr. Borhan and Mr. Nasib from KO-NELAYAN Sabah and the Department of Fisheries Sabah for their cooperation during the study. I would also convey my gratitude to Recaventure Sdn. Bhd. and to Mr. Jackie Ngai from Syarikat Pantai Barat Laut Sdn. Bhd. for his generousity in providing live shrimp specimens during my study. A heartfelt thank to Mr. Juni Jali and Mr. Juni Riong for their assistance in collecting *H. scabra*.

I would also like to acknowledge my friends Mr. Ramlan, Ms. Siti Rahma and Mr. Azlin working for Seaweed Project for their assistance in collecting specimens and providing seaweed for my study.

I also owe my thanks to IPMB lecturers and supporting staffs (Ms. Rossita, Mr. Julian, Dr. Charles, Mr. Asraffuddin, Mr. Amizam, Mr. Bujang, Mr. Ismail, Ms. Siti Badrah, Ms. Rosliah and Mr. Mukti) who have helped me in every way.

Thank you to my dear colleagues and friends; Mr. Yusdi, Ms. Miza, Ms. Norasma, Ms. Zarinah, Mr. Jeremy, Mr. Syuhaime, Ms. Melissa, Mr. Asri, Ms. Salha, Ms. Yuhana, Ms. Faridah, Mr. Ronel, Ms. Bob, Ms. Diha, Bapak Tamrin, Ms. Joanna and Ms. Laura.

Last but not least, I would like to express my heartfelt gratitude to my mother, father, Nikdowi, Esyaron and Neil for their love and emotional support throughout my difficult period.

Finally, with the support and prayer from all of you this thesis is completed.

Alhamdullillah, Praise to Allah.

ABSTRAK

Penghasilan benih timun laut (Holothuria scabra) dalam kaptiviti untuk tujuan akuakultur dan penambahan stok meniadi semakin penting bagi menangani masalah kekurangan stok liar akibat eksploitasi berlebihan. Walaubagaimanapun, kemandirian dan tumbesaran benih dalam kaptiviti adalah tidak konsisten. Kajian-kajian dalam tesis ini dilakukan bagi menyeragamkan sistem kultur untuk memastikan keadaan kultur yang sesuai, tumbesaran lebih cepat dan peningkatan kemandirian bagi benih sebelum aktiviti pembesaran dilakukan secara besar-besaran. Salah satu isu yang ditangani dalam tesis ini adalah kesukaran penentuan biomass yang berpunca daripada kebolehan spesis ini mengubah bentuk badan dan menyimpan serta mengeluarkan sejumlah air dari ruang 'coelomic' semasa pengukuran spesimen hidup. Faktor-faktor penting mempengaruhi kemandirian dan tumbesaran H. Scabra dalam kaptiviti seperti gizi, pertukaran air dan kepadatan stok ditentukan dengan merekabentuk dan menguii kaedah kultur eksperimental yang berlainan. Faktor utama mempengaruhi tumbesaran dalam kaptiviti yang diuji adalah keperluan gizi buatan menggunakan sumber murah dan mudah didapati seperti rumpai laut (Sargassum sp. and Eucheuma sp.) dan nutrien tambahan. Kemungkinan mengurangkan kos serta menambah kecekapan pengeluaran melalui kultur bersama udang (Penaeus monodon) dengan kombinasi gizi dan kepadatan stok yang berlainan, serta sangkar laut juga diuji, Kecekapan rumpai laut (Eucheuma sp.) sebagai 'biofilter' dalam sistem tertutup juga diambil kira. Kebolehyakinan penganggaran biomass menggunakan ukuran linear didapatkan berdasarkan korelasi yang tinggi dan konsisten antara panjang-berat tanpa air (R²=0.9612) dan panjangberat kering (R²=0.9578), yang mana amat berguna bagi kajian spesimen hidup populasi liar atau dalam kaptiviti. Ujikaji dalam kaptiviti menggunakan rumpai laut dalam gizi, menunjukkan kadar kemandirian dan tumbesaran yang baik. Walaupun, gizi Eucheuma sp. dengan pemangkin kalsium-fosfat memberikan kadar tumbesaran paling tinggi (10.469 mg/hari), gizi mengandungi Sargassum sp. sahaja memberikan kadar tumbesaran yang hampir setanding (p > 0.05), yang mana menjadikan Sargassum sp. pilihan yang lebih baik dan murah. Kebaikan Sargassum sp. dalam diet dibuktikan lebih lanjut dengan keputusan ujikaji kultur bersama dimana kemandirian udang didapati lebih tinggi dalam rawatan menggunakan rumpai laut ini sebagai makanan berbanding dengan gizi berasaskan haiwan (pellet udang dan pumpun) kemungkinan akibat peningkatan immuniti disebabkan peningkatan aktiviti 'prophenoloxidase'. Tumbesaran H. Scabra juga ditingkatkan dengan kehadiran udang (0.738 g/hari) akibat peningkatan kualiti bentik oleh udang, menunjukkan potensi bagi mengkultur bersama kedua-dua spesis ini bagi mendapatkan pulangan yang lebih menguntungkan. Pengkulturan H. Scabra di dalam sangkar laut menunjukkan ianya lebih ekonomi berbanding dengan dalam tangki. Walaupun spesimen dalam sangkar laut menunjukkan kadar kemandirian yang rendah, tumbesaran adalah lebih baik (0.238g/hari) pada kepadatan stok 150g/m². Ini mungkin disebabkan adanya sumber bekalan makanan semulajadi dan pertukaran air berterusan dalam sangkar. Walaubagaimanapun, kualiti air dalam sistem tertutup boleh ditingkatkan dengan 'biofiltration' menggunakan Eucheuma sp. Walaupun tiada peningkatan tumbesaran spesimen dalam tangki dengan rumpai laut (p > 0.05). kadar kemandirian yang tinggi menunjukkan keadaan persekitaran yang sesuai dikekalkan dalam sistem ini. Tahap nutrien terlarut rendah dalam sistem dengan rumpai menunjukkan keberkesanan Eucheuma sp. dalam 'biofiltration'. Kajian ini menunjukkan bahawa kecekapan penghasilan boleh ditingkatkan melalui manipulasi faktor-faktor seperti gizi, kepadatan stok, pertukaran air, ciri-ciri substrat dan kandungan bahan organik, dan melalui polikultur dengan spesis yang bersesuaian.

ABSTRACT

GROWTH AND NUTRITIONAL CONDITION OF SEA CUCUMBER (Holothuria scabra) IN CULTURE SYSTEMS

Producing juveniles of sea cucumber (Holothuria scabra) in captivity for aquaculture and stock enhancement is becoming increasingly important in addressing the problem of depletion of wild stocks due to overeharvesting. However, there is inconsistency in survival and growth of juveniles reared in captivity. This work was motivated by the need to standardize the rearing systems to ensure high condition. faster growth and better survival of the juveniles before their grow-out on a large scale. One of the issues addressed in this thesis is the difficulty to measure biomass of the sea cucumber due to its ability to change body shape and to store or expel various volumes of water from the coelomic cavity when measured alive. Factors influencing survival and growth of H. scabra in captivity such as feed, water exchange and stocking density were also determined by designing experimental culture methods. The most important factor crucial to grow-out in captivity investigated was artificial feeding using cheap and abundant dietary stuffs such as the seaweeds (Sargassum sp. and Eucheuma sp.) and supplements. The possibility of reducing cost and increasing production efficiency by co-culture with shrimp (Penaeus monodon) with different combinations of diet and stocking density, and the results rearing in sea pen were also determined. The efficiency of seaweed (Eucheuma sp.) as a biofilter in a closed system was also investigated. The reliability of the biomass estimation technique using the linear measurement was established based on the high correlation obtained consistently between length-dewatered weight ($R^2=0.9612$) and length-dried weight (R^2 =0.9578), which is useful for studying live animals in the wild or in captivity. Trials in captivity using seaweed as diet, revealed good survival and growth. Although Eucheuma sp. diet with calcium-phosphate enhancer gave highest growth rate (10.469 mg/day), the diet comprising Sargassum sp. alone resulted in almost equivalent rate of growth (p > 0.05), which makes Sargassum sp. a better and cheaper option. The benefit of using Sargassum sp. as feed for H. scabra was further proven in co-culture trial where shrimp survival rate was notably higher in treatments utilizing this seaweed compared to animal based feed (shrimp pellet and bloodworm) which might be due to enhanced immunity by prophenoloxidase activity. In addition, growth of H. scabra was improved by the presence of shrimp (0.738 α /day) which may be due to improvement of benthic conditions by shrimp, suggesting the possibility of co-culturing both species for better economic returns. Grow-out of H. scabra in sea pen was found to be more economical than in tanks. Although animals kept in pens showed poor survival percentage, growth was better (0.238 g/day) at stocking density 150g/m². This might be due to reasons such as better growing opportunities due to continuous supply of natural food items and water exchange in pens. However, the improvement of water quality in a closed system was found to be achievable by biofiltration method using seaweed. Though there was only marginal advantage in term of growth of H. scabra (p > 0.05), the high survival rate in the treatment with seaweed implies that a conducive environmental condition was maintained in the closed system. The lower dissolved nutrient level in tank with seaweed showed efficient biofiltration by Eucheuma sp. The study showed that the efficiency of production could be increased by manipulating factors such as feed. stocking density, water exchange, substrate features and organic matter content, and by polyculture with compatible species.

ABBREVIATIONS

cm	centimeter
FAO	Food and Agriculture Organization of the United Nations
9	gram
g/day	gram per day
g/m ²	gram per meter square
g/m ³	gram per meter cube
m	meter
mg	milligram
mg/l	milligram per liter
mg/day	milligram per day
ml	milliliter
nm	nannometer
hà	microgram
°Č	degree Celcius
%	percentage



TABLE OF CONTENTS

	PAGE
TITLE	1
	iii
	iv
ABSTRAK	v
ABSTRACT	
ABBREVIATIONS	VI
CONTENTS	vii
LIST OF FIGURES	ix
LIST OF TABLES	×
CHAPTER 1: INTRODUCTION	1
1.1 SEA CUCUMBER 'SANDFISH' Holothuria scabra	1
1.1.1 ECONOMIC IMPORTANCE	1
1.1.2 ECOLOGICAL IMPORTANCE	2
1.2 H. scabra POPULATION AND FISHERY IN SABAH	3
1.3 JUSTIFICATION FOR STUDY ON H. scabra	4
CHAPTER 2. LITERATURE REVIEW	7
2.1 SEA CUCUMBER RESEARCH AND AQUACULTURE	7
	9
2.3 REPRODUCTION AND RECRUITMENT	10
2.4 DIET AND FEEDING BEHAVIOUR	11
2.5 PARASITES AND DISEASES	13
2.6 SURVIVAL AND GROWTH	14
2.7 MANAGEMENT OF SEA CUCUMBER FISHERY	15
2.8 STOCK ENHANCEMENT	16
	19
CHAPTER 3: MATERIALS AND METHODS	
	20
3.1.1 PROCUREMENT OF TEST SPECIMENS	20
3.1.2 PREPARATION OF SPECIMENS	21
3.1.3 LENGTH AND WEIGHT MEASUREMENTS	21
3.1.4 LENGTH-WEIGHT KELATIONSHIP CALCULATION	22
3.2 EXFERIMENT Z 3.2.1 SELECTION OF JUN/ENJUE H cooper AND	23
TRANSPORTATION	23
322 ACCLIMATIZATION	24
323 CULTURE SYSTEM	24
3.2.4 DIFTARY TREATMENTS FOR THE JUVENILES	25
3.2.5 SURVIVAL AND GROWTH	27
3.2.6 STATISTICAL ANALYSIS	28
3.2.7 SEDIMENT ANALYSIS	28
3.2.7.1 ORGANIC MATTER ANALYSIS	28
3.2.7.2 PARTICLE SIZE FRACTIONATION	29
3.2.8 WATER QUALITY MONITORING	30

3.3 EXPERIMENT 3	30 31
Monodon SPECIMENS	51
3.3.2 ACCLIMATIZATION OF <i>H. scabra</i> AND <i>P. monodon</i> SPECIMENS	31
3 3 3 CILI TURE SYSTEM	32
3.3.4 FEEDING TREATMENTS	32
3.3.5 WATER QUALITY MONITORING	34
3.3.6 SEDIMENT ANALYSIS	34
3.3.7 H. scabra SURVIVAL AND GROWTH	34
3.3.8 P. monodon SURVIVAL AND GROWTH	34
3.3.9 STATISTICAL ANALYSIS	34
3.4 EXPERIMENT 4	35
3.4.1 COLLECTION AND TRANSPORTATION OF H. scabra	35
SPECIMENS	
3.4.2 ACCLIMATIZATION	35
3.4.3 TREATMENTS IN DIFFERENT CULTURE SYSTEMS	36
3.4.3.1 SEA PEN	36
3.4.3.2 FLOWTHROUGH SYSTEM TANK	36
3.4.4 ENVIRONMENTAL DATA COLLECTION	36
3.4.5 SURVIVAL AND GROWTH	37
3.4.6 STATISTICAL ANALYSIS	37
3.5 EXPERIMENT 5	37
3.5.1 PREPARATION OF <i>H. scabra</i> SPECIMENS	37
3.5.2 CULTURE SYSTEM	37
3.5.3 WATER QUALITY MONITORING	38
3.5.3.1 DETERMINATION OF NITRITE	39
3.5.3.2 DETERMINATION OF NITRATE	40
3.5.3.3 DETERMINATION OF AMMONIA	42
3.5.3.4 DETERMINATION OF PHOSPHATE	43
3.5.4 SURVIVAL AND GROWTH	45
3.5.5 STATISTICAL ANALYSIS	45
CHAPTER 4: RESULTS UNIVERSITI MALAYSIA SABAH	46
41 EXPERIMENT 1	46
4.2 EXPERIMENT 2	47
4.3 EXPERIMENT 3	49
4.4 EXPERIMENT 4	52
4.5 EXPERIMENT 5	56
	59
	50
5.1 LENGTH-WEIGHT RELATIONSHIP	59
5.2 UTILIZATION OF SEAVEEDS AND CELL SALT IN A. SCADIA DIET	00
5.3 INTEGRATION OF P. MONODON AND H. SCADIA CULTURE	00
DENIQ	09
	72
CUITURE	12
	75
CHAPTER 6: CONCLUSION	
REFERENCES	79

LIST OF FIGURES

		PAGE
Figure 3.0	Map of Sabah showing sampling location of <i>H. scabra</i> (Balambangan Island) and location of Borneo Marine Research Institute (BMRI).	19
Figure 3.1(a)	H. scabra placed in a plastic tray.	20
Figure 3.1(b)	H. scabra covered with a wet towel (soaked with seawater).	20
Figure 3.2	Tanks equipped with aeration system used in the conditioning process.	21
Figure 3.3	Gut of H. scabra consisting of fine sediments.	21
Figure 3.4	Oven dried H. scabra.	22
Figure 3.5	<i>H. scabra</i> in a hatchery in Tuaran owned by a private company (Recaventure Sdn Bhd).	24
Figure 3.6	Small juveniles placed in experimental tray equipped for aeration by means of air stones.	25
Figure 3.7(a)	Fresh Eucheuma sp.	26
Figure 3.7(b)	Grinded Eucheuma sp.	26
Figure 3.8(a)	Fr <mark>esh Sarg</mark> assum sp.	26
Figure 3.8(b)	Grinded Sargassum sp.	26
Figure 3.9	H. scabra juvenile.	31
Figure 3.10	P. monodon post larva. IVERSITI MALAYSIA SABAH	31
Figure 3.11	Flowthrough experimental tank system.	32
Figure 3.12	Closed re-circulating system.	38
Figure 4.1	Linear regression graph for length and de-watered weight in <i>H. scabra</i> .	46
Figure 4.2	Linear regression graph for length and dry weight in <i>H.</i> scabra.	47
Figure 4.3	H. scabra with skin lesions covered with mucus.	48
Figure 4.4	Weekly survival percentage of <i>H. scabra</i> in sea pens (T1 and T2) and tanks (T3 and T4).	53
Figure 4.5	Weekly growth of <i>H. scabra</i> in sea pens (T1 and T2) and tanks (T3 and T4).	53
Figure 4.6	Nutrient concentration in tank without seaweed (T1).	57
Figure 4.7	Nutrient concentration in tank with seaweed (T2).	58
Figure 5.1	Typical sea pen for grow-out of <i>H. scabra</i> in Kudat.	69

LIST OF TABLES

		PAGE
Table 1.1	Taxonomic classification of H. scabra.	1
Table 2.1	H. scabra performances without additional feeding at different conditions.	15
Table 3.1	Composition of test diets.	25
Table 3.2	Summary of treatments.	33
Table 3.3	Summary of trials with different culture methods and stocking densities.	36
Table 4.1	Juvenile survival (%) and growth (g/day) in different dietary treatments.	48
Table 4.2	Survival (%) and growth (g/day) of <i>H. scabra</i> and <i>P. monodon</i> .	51
Table 4.3	Survival (%) and growth (g/day) of <i>H. scabra</i> in sea pens and tanks.	52
Table 4.4	Water parameters for Malholom Bay during trial period.	54
Table 4.5	Water parameters for Balambangan Island.	54
Table 4.6	Organic matter content and type of sediments.	55
Table 4.7	Particle size fractions (%) in the sediment.	56
Table 4.8	Survival (%) and growth (g/day) of <i>H. scabra</i> in closed re- circulating tank.	57

CHAPTER 1

INTRODUCTION

1.1 Sea Cucumber 'Sandfish', Holothuria scabra

More than 1200 species of sea cucumbers (Holothuridae) have been identified around the globe, with currently about 30 species known to be harvested (Purwati, 2005). Of the 1200 holothurians, *Holothuria scabra*, generally known as sandfish (or popularly called 'balat' by local people of Sabah) is the most valuable and sought after species. Taxonomic classification of *H. scabra* is described in Table 1.1.



Table 1.1: Taxonomic classification of H. scabra.

1.1.1 Economic Importance

The world sea cucumber market is largely controlled by the Chinese traders (FAO, 1990; Conand, 2001). The dried form of *H. scabra* (Trepang or Beche-de-mer) supports a multimillion dollar industry for a number of countries in the Asia-Pacific region such as the Phillipines, Vietnam and the Solomon Islands. It is also an important income generator for the downtrodden sections of the local community living in the remote coastal areas of Sabah. *H. scabra*, is popular for its culinary

qualities. When processed or dried it can fetch a high price in the Sabah local markert (RM 200 to 400 per kilogram). Due to this reason, *H. scabra* has been a target species for fishermen.

Concomitant with the increase in demand, there is increase in fishing pressure, and hence concern about overexploitation of this species. Based on the statistical records by Department of Fisheries Sabah from 2003 to 2004, the significant decline (39.10 %) in total landings of sea cucumbers led to increase in its retail and wholesale prices by about 3 % within a one-year period.

1.1.2 Ecological Importance

H. scabra is a detritivore that feeds upon organic matter which accumulates at the seabed and mixes with the sediment. The most significant benefit of *H. scabra* to the benthic ecosystem is probably the bioturbation of sediments (Hamel *et al.*, 2001). Sea cucumber activities such as burrowing, ingestion, and defecation of sediment grains increase sediment aeration and influence contaminant equilibria with the overlying water. It is also believed that the burrowing habit of *H. scabra* in seagrass beds has positive influence on the production and density of this marine critical habitat (Uthicke, 2004). Moreover, the sediment-water interface increases in area, affecting chemical fluxes and thus the exchange between the seabed sediment and the water column (Gerino, 1990; Austen *et al.*, 2002). Experiments conducted on deposit feeder, *Stichopus japonicus*, by Michio *et al.* (2003) in an enclosed area found that the algal biomass and organic matter concentration of seabed sediment decreased in the presence of sea cucumber.

Inhibition of algal bloom in coral reef area by sea cucumber has also been noticed by Moriarty (1982), and Uthicke (1999) who showed that when kept in densities above natural level, algal biomass was reduced significantly. The

microalgae appear to benefit from the enhancement of nutrient level from holothurians' excretion (Uthicke, 2001b). Removal of these holothurians can have negative effects on reef ecosystem (Uthicke, 2004).

1.2 *H. scabra* Population and Fishery in Sabah

H. scabra is listed in CITES (Commission on International Trade of Endangered Species of Flora and Fauna) (Appendix II). This appendix includes species that, although not threatened with extinction, may become at least locally extinct unless trade is strictly regulated. This means, international trade may be allowed, but strictly controlled, and the CITES member country or party may only grant a permit to export the animals after it has determined that the export would not be detrimental to the survival of the species (Bruckner and Field, 2003).

In Sabah, the main method for collecting *H. scabra* is by hand during lowtide, when animals are exposed and easily seen. They are also collected by diving or snorkeling, and most of the large animals are caught as by-catch of deep-sea trawlers. Their ease of collection, which does not require sophisticated fishing techniques has led to overexploitation, and decline in wild population, in many places below replacement level. Thus, the wild population of this target species may be unable to rebound.

Historically, Sabah has been the only state in Malaysia with a relatively important sea cucumber fishery industry. Based on the statistical records of sea cucumber landings by the Department of Fisheries Sabah (2002, 2005, and 2006) and reports by Baine and Sze (1999) and Sze (2004), important fishing areas for sea cucumbers in Sabah are Kudat, Semporna, Sandakan, Kota Kinabalu, Kota Belud and Kota Marudu. In the year 2000, the total fisheries value constituted by sea cucumbers in Sabah was 0.17 %. Sempoma, located on the east coast of Sabah, was the most important sea cucumber fishing area, contributing some 62 % of the total landings of sea cucumbers in the year 2000, followed by Sandakan (14 %), Kota Kinabalu (13.66 %), and Kudat (9.99 %) (Department of Fisheries Sabah, 2002; Sze, 2004).

Records by Department of Fisheries Sabah from 2003 to 2004, showed significant decline in landings (39.10 %) in total landings of sea cucumbers. Kota Marudu and Kota Belud, which used to have record sea cucumber landings in the years before, disappeared from the landing statistical record in 2004 (Department of Fisheries Sabah, 2005, 2006).

1.3 Justification for Study on *H. scabra*

Due to importance of *H. scabra* as an income generating resource for the poor communities living in the remote coastal areas of Sabah, legislating the collection methods and ceiling on their capture level may not be very effective. This study provided a basis for the initiation of *H. scabra* re-stocking or stock enhancement programme in Sabah for commercial, subsistence or conservation purposes. In addition, it examined the factors that are crucial to the maintenance of grow-out in captivity using accessible technology in a cost-effective manner. One of the most important factors investigated was the nutritional needs of *H. scabra* using cheap and abundant resources such as the seaweeds (*Sargassum* sp. and *Eucheuma* sp.). Most of the research on nutritional requirements of *H. scabra* was done for the larval stage of the animal. There is lack of information on nutritional management at juvenile stage should be given due importance as this stage of growth is as critical to their survival as in larval stage. Plant derived proteins such as in seaweed is a potentially good source of protein for grow out of juveniles as it contains high protein level and have

been proven to be cost-effective and a good partial substitution of fish meal, in fish grow out (Davies *et al.*, 1997). Apart from plant derived protein, the suitability of animal based feed should also be looked into as comparison. Therefore, study on these readily available resources as a potential commercial feed for *H. scabra* is worth looking into in order to minimized cost of rearing as well as the rearing period.

The potential of combining the culture of *H. scabra* with other marine organisms was also determined as a means of improving production efficiency consistent with the organic farming criteria. The polyculture concept was given due importance in this investigation. It was based on the criteria of combining multiple types of organisms which are compatible and whose composite farming increases yield/unit area. Co-culture of sea cucumber and shrimp was organized on experimental basis. Waste output from intensive shrimp farming (uneaten feed, faeces, dissolved nutrients, dissolved organic compounds, chemicals and therapeutics) produces adverse impact on the environment (Beveridge *et al.*, 1994; Briggs and Funge-Smith, 1994; Lorenzen *et al.*, 1997; Páez-Osuna, 2001). The treatment of dissolved nutrient effluents resulting from an aquaculture farm such as the shrimp farm often involves higher degree of technology and, therefore it would be too expensive to implement for most farmers (Troell *et al.*, 1999). Hence, the remediation of waste in the form of dissolved nutrients in seawater is often being ignored.

One possible way of defraying the cost of growing *H. scabra* would be to combine the culture of this species with shrimp, thereby saving the costs of constructing and managing rearing ponds exclusively for this holothurian. This may be a possibility for farms dedicated to rearing *Penaeus monodon* because juvenile sandfish is one of the few species of sea cucumbers that can tolerate a reduction in salinities as low as 20 % (Mercier *et al.*, 1999). The ideal range of water parameters for culture of *H. scabra* in the hatchery are temperature 27 – 29°C; salinity 26.2 –

32.7 ‰; dissolved oxygen 5 – 6 mg/l; pH 6 – 9 and ammonia 0 mg/m³, but not exceeding 70 – 430 mg/m³ (Battaglene and Bell, 1999). The ideal range of water parameters for *P. monodon* culture are temperature $28 - 33^{\circ}$ C; salinity of 15 - 30 % (this species is known to be able to survive nearly freshwater condition to up to 35 ‰); dissolved oxygen of more than 4 mg/l; and pH 7.8 – 8.5 (Rosly, 1990; Sabah Fisheries Department, 1991; Annita, 2003). Comparison between the optimum environmental conditions of the sea cucumber and the shrimp species showed that there is distinct possibility that both tolerate each others' rearing conditions.

In addition to producing an additional crop, such a co-culture may also have other advantages for shrimp farmers. These potential benefits stem from the burrowing and feeding behaviour of *H. scabra*. Burrowing by *H. scabra* may help to mobilize nutrients from the sediment into the water column, improving the normally low productivity of ponds early in the shrimp production cycle. The feeding and burrowing behavior of *H. scabra* may also help assimilate excess nutrients and bacteria towards the end of the production cycle, when eutrophication of pond water causes concern (Uthicke, 2001b). This study was designed to present information on growth of *H. scabra* in tanks and sea pen, and practical feasibility of its co-culture with the tiger shrimp.

CHAPTER 2

LITERATURE REVIEW

2.1 Sea Cucumber Research and Aquaculture Development

Realization of the increase in demand coupled with decline in total landings of sea cucumbers since mid 1980s' prompted the initiation of research on commercial species such as *H. scabra* oriented towards their conservation and commercial production (Morgan and Archer, 1999). Main fields of interest are ecology (habitat, distribution, survival, growth and reproduction cycle) and induced spawning (Conand, 1993; Hamel *et al.*, 2001; Purcell, 2004; Asmizah, 2006).

Survival and growth of *H. scabra* in the wild has been difficult to measure due to their cryptic behavior and difficulty in tagging. Researchers have been experimenting and developing tagging methods for studying the survival and growth for the last 25 years (Harriott, 1980; Shelley, 1981; Yanagisawa, 1995; Morgan, 2000). Some tagging methods developed for this purpose include:

1) Use of fluorescent dye to stain the calcareous plates surrounding the buccal cavity of the sea cucumber (Harriott, 1980).

2) Use of a red hot wire in branding (Yanagisawa, 1995)

3) Use of a t-bar tag in which a tagging gun is usually used to insert a small t-bar tag into the body wall of sea cucumber. However, in most cases a localized necrosis of tissues occurred and the tags fell out or entered the coelomic cavity of the sea cucumber within a few months. This tagging method would not be suitable for a long-term study, however it may still be adequate for tagging animals in captivity for a short period of time (Shelley, 1981; Morgan, 2000).

Restocking of H. scabra with hatchery reared juveniles makes sense when conservation efforts, such as establishment of marine parks (Basiron and Zakariah, 2004: Sze. 2004), implementing moratorium (Baine, 2004; Bell and Warwick, 2004; Gamboa et al., 2004), prohibiting collection using compressed air (Alfonso et al., 2004; Aumeeruddy and Payet, 2004), limiting collection to certain size range and fishing quota (Lawrence et al., 2004; Mmbaga and Mgaya, 2004) fail to stem the decline in wild stocks. However, before a stock replenishment programme through induced spawning techniques can be developed, the reproductive cycle of these animals has to be studied to ensure successful captive breeding. Natural reproductive cycle of H. scabra has been examined in Australia, New Caledonia, Papua New Guinea, the Solomon Islands and Malavsia (Conand, 1993; Battaglene and Bell, 1999; Asmizah, 2006). Spawning success in this species was found to depend mainly on seasonal maturity and lunar periodicity. Based on this knowledge, combined with the utilization of induced spawning techniques, the success rate of captive breeding can be increased. Induced spawning techniques that have been developed for *H. scabra* include: 1) thermal stimulation in which the temperature is raised by 3 to 5 °C for about one hour; 2) water jet technique; and 3) addition of commercially available powdered algal product, Schizochytrium sp., Algamac-2000 into the water column. The thermal stimulation technique has proven to be the most reliable method for inducing H. scabra to spawn, and it is recommended that this type of stimulation should be combined with the addition of Algamac dried algae (Hamel and Mercier, 1996a).

The study on *H. scabra* ecology is essential for the development of its aquaculture either for conservation or commercialization purposes (Conand, 2004).

Studies have also been done on the transportation method of sea cucumber (Battaglene et al., 2002; Purcell, 2004; Xiyin et al., 2004; Purcell et al., 2006).

Battaglene *et al.* (2002) examined the results of stress such as evisceration and premature spawning of ripe *H. scabra* individuals during the transport of broodstock to the hatchery. Stress was found to be linked to the animals' sensitivity to rapid change in water temperature, salinity, and pressure. As a result of this study, these authors have suggested a more effective method of transporting *H. scabra* by plane for periods of up to 10 hours, where animals are packed individually in plastic bags within a single insulated box. Another study by Purcell *et al.* (2006) found that hatchery produced *H. scabra* proved to be hardy for transport in high densities (100 to 200 juveniles per bag) as long as the seawater is held at a moderately low but constant temperature. Cooler temperatures result in decline in oxygen consumption, and hence a reduction in oxygen related stress.

2.2 Habitat

H. scabra inhabits shallow, sandy coastal habitat (Mercier *et al.*, 2000b). Observations on habitat of this species in the coastal waters of Kudat by Melissa (2001) showed preference of this species to an average pH of 8.74, low turbidity averaging 5.5 NTU, and a mean temperature of 28.3°C.

Based on the recent sea cucumber landing record of the Department of Fisheries Sabah (2004), most of the commercial level abundance of the sea cucumber in Sabah can be found in Kudat, Semporna, Sandakan, Kota Kinabalu, Kota Belud and Kota Marudu. However, this record is very general and based on total sea cucumber landings regardless of their species.

The recruitment of *H. scabra* larvae occurs in certain habitats. The juveniles migrate to 'adult habitats' later in life. The settlement of *H. scabra* occurs in shallow seagrass beds (Mercier *et al.*, 2000b). Distribution patterns suggests that they move to deeper areas later (Hamel *et al.*, 2001). Adult habitats may offer better foods but a

higher risk to predators, or may not have suitable substrata for larval settlement. Moreover, juveniles survive and grow better in certain micro-habitats within the general habitat in which they occur along with adults. Observations made by Melissa and Saleem (2001) revealed that there was no significant size-related microhabitat shift between the widely different sizes of sea cucumbers in Kudat. Small *H. scabra* were usually found in the shallow area of a sheltered coast, bay or estuary, and particularly in mangrove or seagrass beds (Mercier *et al.*, 2000b). Studies on their distribution showed that the adults prefer deeper water where food availability is higher even though there is higher risk of predation. According to Tyler and Banner, (1977) and Sloan and Von Bodungen (1980), the most important factor influencing their distribution is coastal perturbation, as hydrodynamics could be a modifying factor for sediment granulometry and larval dispersion (Massin and Doumen, 1986).

Based on observations in Solomon Islands, *H. scabra* prefers substrate classified as muddy, with high content of organic matter, generally along the coastal mangrove areas. This animal has been observed to avoid very fine sediment texture that would not provide stability for easy burrowing (Mercier *et al.*, 2000b).

Mercier *et al.* (2000b) found that *H. scabra*, especially the juveniles inhabited seagrass bed. There was no correlation with the type of seagrass and their distribution. It is often seen to occur in a few types of seagrass beds such as those of *Enhalus* sp.

2.3 Reproduction and Recruitment

The reproductive cycle of *H. scabra* is influenced by environmental stimuli such as temperature and abundance of food supply (Chia and Walker, 1991). Several studies on reproductive cycle of sea cucumber suggested that gamete maturation was influenced by physical and chemical factors such as lunar cycle, photoperiod, food

supply and temperature. These factors act together, successively or independently of one another (Hamel and Mercier, 2004). A study on the natural reproductive cycle of *H. scabra* in Sabah by Asmizah (2006) showed a characteristic continuous asynchronous gametogenesis that began in March, with major spawning period in May and minor spawning pulses in June and August. The continuous gonad development of *H. scabra* in Sabah was similar to that recorded in the Solomon Islands and the Phillipines (Ong Che and Gomez, 1985 and Ramofafia *et al.*, 2003).

2.4 Diet and Feeding Behaviour

The feeding biology of sea cucumbers has been examined *in situ* (Cameron and Fankboner, 1984; Ahlgren, 1998; Uthicke and Karez, 1999). The total organic matter content in the substrate that represents plant, animal and microbial materials in various stages of decomposition, is the main dietary component that influences the growth of the sea cucumber (Keesing and Halford, 1992; Summer, 2000). The optimum range of organic matter in seabed habitat preferred by *H. scabra* is 5 to 10 % (Mercier *et al.*, 2000b). Though there is a strong link between habitat rich in organic matter (>10%) and preferred habitat for *H. scabra*. Mercier *et al.* (2000b) also found that these animals avoid very rich substrata containing more than 30 % of organic matter, which can possibly be anoxic in the first few millimeters.

In a study on other species of sea cucumbers and their effect on an ecosystem, Klinger *et al.* (1994) observed that Aspidochirotida (*Holothuria atra*, *Holothuria leucospilota*, *Holothuria edulis* etc.) in the lagoon and reef flat of Heron Island, Great Barrier Reef utilized 3.93 (0.06 %) and 12.76 g m²/day (0.22 %) of surface sediment, respectively. Wiedemeyer, (1992) and Mercier *et al.*, (1999) found that a small percentage of available surface sediment consumed by these animals denoted slight competition between co-existing Holothuroidea in the wild in terms of

competition for food. This is due to their low level in the food chain, where they feed by processing organic matter, bacteria, diatoms, cynophyceans and foraminiferans.

Optimum ingestion and digestion activities are reliant on the mobility of the animals, which is dependent on energy and food availability (Hammond, 1982). In one of his experiments, Battaglene (1999) mentioned that food availability could be a limiting factor in survival of hatchery reared juvenile *H. scabra*. The availability of food in the holding tank, boundary effects, photoperiod and temperature regimes may also influence the activity pattern and feeding behaviour of *H. scabra*. The effect of fine scale regulation of environmental parameters in captivity on feeding behaviour and egg production, in combination with species-specific feeding processes, is likely to be important in obtaining successful spawning. Further, the type of diet may not be as critical as the bacterial and fungal communities that the diet sustains.

Seaweed has varied protein content, with highest concentration in red algae (Rhodophyta), ranging from 30-40 % of dry weight, followed by up to 20 % of dry weight in green algae, and 5-11 % in brown algae. Davies *et al.* (1997) reported that a partial substitution of nutritionally superior fish meal with seaweed (*Porphyra purpurea*) given to thick-lipped grey mullet (*Chelon labrosus*) proved to be cost-effective. In a grow out experiment, good growth results were obtained when finely chopped or pasted *Sargassum* sp. were used to feed juvenile *H. scabra* in captivity (Rasolofonirina *et al.*, 2004). Due to their abundance and high protein content it would be practical to utilize cheap plant derived proteins, such as seaweeds in *H. scabra* grow out.

At present, no study has determined how the regulation of exogenous cues and its effect on an endogenous rhythm influences feeding and reproduction of sea cucumbers in captivity. Information explaining how the captive environment affects