INDUCED SPAWNING, LARVAL REARING AND JUVENILE GROWOUT OF SEA CUCUMBER (*Holothuria scabra*) IN KUDAT

BY

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ABSTRACT

Severe overfishing and stock depletion of worldwide sea cucumber has encouraged the development of sea cucumber aquaculture. Five species of local microalgae were cultured prior to larval and juvenile rearing as the main food source. Results for mass culture showed that they can be cultured outdoor which yielded a higher volume production than indoor culture. Culture in polythene bag showed a significant difference in growth compared to carboy culture and subsequently resulted in shorter harvest period. Adult *H. scabra* were collected from wild and subjected to two spawning induction method namely the thermal stimulation and dry-algae stimulation. Thermal stimulation was proven to be the best method by producing about 1.7 million eggs. Early auricularia larvae were observed 48 h after fertilization and were fed on mix microalgal culture. Auriculariae larvae transformed into dolilaria larvae after 11th day fertilization and the non feeding doliolaria metamorphosed into creeping pentactulae after 17th day. The survival rate of 4.2 % was achieved. Juveniles were fed with mixture of microalgae, sea weed and spirulina according to their growth stages. Average growth rate was 0.8 mm per day. Survival rate in the juvenile rearing tank was 32%. After 60 days of tank cultivation, juveniles reached 20 mm and were transferred either to hapanet or outgrow pond at 200 animals/m^2. Total loss was observed in the hapanet while good growth was observed in the outgrow pond with survival rate of 36%. Juveniles reached average weight of 270 g after 3 months of outgrow pond cultivation. Growth was generally inversely proportional to the stocking density. Higher survival rate and better growth was observed in pond with lesser stocking density.

خلاصة البحث

أجبر الإفراط شديد في صيد خيار البحر واستنزاف مخزونه في جميع أنحاء العالم إلى تنمية وتطوير الاستزراع المائي لخيار البحر. تم استنبات 5 أنواع من الطحالب المحلية كمصدر للغذاء قبل تربية خيارات البحر اليافعة ويرقاتها. أظهرت نتائج الاستزراع المكثف أنه من الممكن استزراع خيار البحر في الخارج وبمحصول انتاجي أكبر من الاستزراع الداخلي في الأماكن المغلقة. أظهر الاستزراع في أكياس البوليثين فارقا ملحوظا في النمو مقارنة بالاستزراع في الدامجانات الزجاجية مسفرة عن فترة حصاد أقصر. تم جمع كبار الهيلوثريا سكابرا (*Holothuria scabra*) *بريا وتعريضها للتفريخ الصناعي بطريقة التحفيز الحراري والتحفيز بالطحالب الجافة. التحفيز الحراري كان الأكثر تأثيرا، منتجا حوالي 1.7 مليون بيضة. تحولت اليرقات الأذينية* (Auriculariae larvae) إلى يرقات برميلية (dolilaria larvae) في اليوم الحادي عشر بعد التلقيح، وتحولت اليرقات البرميلية العديمة التغذية (non-feeding doliolaria) إلى يرقات خماسية اللوامس (creeping pentactulae) في اليوم السابع عشر. كان معدل البقاء على قيد الحياة حوالي 4.2%. تم إطعام خيارات البحر اليافعة مزيجا من الطحالب، والأعشابا البرية، والسبيرولينا وفقا لمراحل نموها. كان معدل النمو حوالي 0.8 ملم في اليوم. معدل البقاء على قيد الحياة في خزان خيارات البحر اليافعة كان 32%. بعد 60 يوما من الاستزراع في الخزانات، وصل طول خيارات البحر اليافعة إلى 20 ملم و نقلت بعد ذلك إلى أقفاص هابا الشبكية و إلى البرك المفتوحة بكثافة معدلها 200 حيوان لكل متر مربع. لوحظت الخسارة الكلية في أقفاص هابا الشبكية، أما في البرك المفتوحة فكان النمو جيدا بمعدل بقاء حوالي 36%. وصل متوسط وزن خيار البحر اليافع إلى 270 غم بعد 3 شهور من الاستزراع في البرك المفتوحة. بشكل عام، تناسب النمو عكسيا مع كثافة التخزين، وقد لوحظ ارتفاع معدل البقاء على قيد الحياة والنمو الأفضل في البرك ذات كثافة تخزين أقل.

APPROVAL PAGE

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

°C	Degree Celcius
µm	Micrometer
cm	Centimeter
d	Day
EDTA	Ethylenediaminetetraacetic acid
g	Gram
h	Hour
ha	Hectare
ind	Individual
KCl	Potassium chloride
Kg	Kilogram
L	Liter
m^2	Meter square
m^3	Meter cube
min	Minute
mL	Milliliter
mm	Millimeter
ppm	Part per million
ppt	Part per trillion
PVC	Polyvinyl chloride
sp.	Species
w/w	Weight per weight

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Sea cucumbers are sea invertebrates from the phylum Echinodermata class Holothuroidea. They live in a variety of habitat, but are most diverse on tropical shallow-water coral reefs in all tropical and temperate oceans (Liao, 1997; Massin, Zulfigar, Tan Shau Hwai & Rizal Boss, 2002; Rasolofarina et al., 2004; Hearn & Pinillos, 2006). Sea cucumbers are well known as a source of medicine in the treatment of hypertension, asthma, rheumatism, cuts and burns, impotence and constipation (Chen, 2003; Wen, Hu & Fan, 2010). In Malaysia, research on sea cucumber has started since 1980s focusing on the aspects of consumption, folklore belief, ecology, reproduction and medicinal value (Ridzwan, 2007).

The never ending potential in every aspect of sea cucumber fisheries has caused it to decline in number in the wild due to over exploitation (Choo, 2008). The exploitation of sea cucumber begin since over thousand years ago particularly around Southeast Asia and Pacific. Species exploited for food include the *Holothuria scabra*, *H. nobilis*, *H. fuscogilva*, *H. fuscofunctata*, *H. leucospilota*, *Bohadschia graeffei*, *Bohadschia marmorata* and *Thelenota ananas*. The market demand goes beyond global supply, and prices are rising, particularly for more in-demand species of sea cucumber. Sea cucumber harvesting most typically follows a boom-and-bust pattern of exploitation (Carleton, Hambrey, Govan, Medley & Kinch, 2013). The market makers, on the other hand, are struggling to find new sources of supply, and the market is facing a declining rate in the volume of global supplies. Coastal communities also rely heavily on sea cucumber harvesting, because it is one of their few sources of cash income.

Thus, following the overexploitation in the wild, sea cucumber fisheries was introduced with ‘sea cucumber ranching’ or ‘stock enhancement’ method (Bell and Nash, 2004). These methods have been practiced in Japan for more than 30 years ago. Stock enhancement plan involved spawning of sea cucumbers using several methods. Spawning in tropical and temperate sea cucumbers are usually induced through the application of short term environmental stresses (Battaglene, Seymour, Ramofafia & Lane, 2002). Environmental factor thought to be involved include the temperature, light intensity, photoperiod, salinity, tidal flux, food availability and change in food type. Thermal stimulation is a common method of inducing spawning in many invertebrates. However, reliable techniques for induced spawning have been developed such as algal stimulation, water-jet stimulation and gonad stimulation (Ajith Kumara, Jayanatha, Pushpakumara, Bandara & Dissanayake, 2013). So far the several methods of spawning induction have been successful in some commercial holothurians, which offer an alternative to harvesting wild populations.

Juvenile and adults of sea cucumbers feed on detritus or decaying organic matter. Under captive or culture conditions, they can feed on dry sea weed or artificial feed. During larval stages and early juvenile, they were fed with mix microalgae. The practice of feeding live microalgae is essential especially during larval stages and this is very costly and labor intensive (Shields & Lupatsch, 2012). Efforts have been made to study on ways to reduce the production cost of microalgae culture in sea cucumber hatchery (Iba, Rice & Wikfors, 2014).

Sea cucumbers fishery used to be the main source of livelihood in some areas in Malaysia, especially in Sabah. In the 1980s, sea cucumber landings recorded an annual catch of about 400 – 500 tonnes, while landings in the 2000s fell to an annual catch of around 100 tonnes (Choo, 2008). The high value of sea cucumber species, the ease of harvesting from shallow water and their vulnerable nature contribute to overexploitation in Malaysian waters (Choo, 2004, Choo, 2008).

Prominent commercial sea cucumbers in Malaysia are *Stichopus horrens* and *H. scabra*. The industry based on *S. horrens* trade supports a large number of individuals and promotes Pulau Langkawi, an island on the northern part of West Malaysia, as a centre for production of sea cucumber medicine. Extracts from this species were used widely and known to have antifungal and antibacterial components (Ridzwan, 2007). Overfishing of sea cucumber *S. horrens* in Langkawi has forced the state to outsource it from Adang, Thailand. However, in Pulau Pangkor, *S. horrens* are still found in relative abundance on the reef flats (Choo, 2004). In Sabah, the state supports Malaysia's largest fishery with the main sea cucumber products are beche-de-mer of *H. scabra*. There are quite a number of sea cucumber farm in Sabah that were built by villagers to rear sea cucumber particularly *H. scabra*.

Sea cucumber breeding in Malaysia lacks advanced technology and is mainly carried out as home-based industries. There is no commercial hatchery built solely for sea cucumber export or stock enhancement programme. Attempts to raise and produce sea cucumber from an aquaculture system in Malaysia has been scarce, almost nil. Breeding trials for *S. horrens* were made by the Fisheries Research Institute of Malaysia in late 2008 and mid 2009 for restocking purposes but the survival rate in the sea was not satisfactory. Despite the increasing interest of this sea product in the world markets, the only approach has been an unregulated extracting fishery, which has led only to a serious depletion of natural populations of sea cucumber in Malaysia.

1.2 **SIGNIFICANCE OF STUDY**

The present study was undertaken to mass-produced sea cucumber *H. scabra* in three stages: larval culture, juvenile rearing and farming of hatchery produced juvenile in growout pond, hapanet. The sea cucumber was induced to spawn and the best spawning method was chosen and practice throughout the study. Life cycle of *H. scabra* was studied in detail starting from the formation of fertilized egg until it become adult. Development stages of *H. scabra* are important as it determined the duration taken for growth and also the survival rate of the larval and juvenile. During this study, live microalgae were cultured and fed according to the growth stages. Microalgae production is costly and labor intensive, thus method to decrease the cost and increase algae production was also studied. Juvenile growout in sea pen, ponds and hapanet have been reported in many literatures and these methods were compared for the survival rate of the juveniles reared.

So far, to our knowledge, no attempt has ever been made to captively breed *H. scabra* in Malaysia. Furthermore, currently there is no sea cucumber aquaculture company in Malaysia that is able to mass produced *H. scabra* up to a marketable level and retail produced the products. Thus, this hatchery aims to produce a premium high quality *H. scabra* and sell it to the retailers. Sea cucumber aquaculture is feasible and that it could be used to contribute to the restoration of depleted natural populations and allow, in due course, a sustainable fishery.

1.3 OBJECTIVES

1.3.1 General Objective

To mass-produced sea cucumber *H. scabra via* induced spawning and subsequently cultivate the larvae and juvenile in tank, outgrow pond, hapanet.

1.3.2 Specific Objectives

1. To isolate five species of microalgae and study the effects of culturing method (carboy culture and polythene bag culture) on the growth of microalgae isolated from the coastal area of the sea cucumber hatchery.
2. To study and compare the two methods (thermal induction and dried algal induction) for sea cucumber spawning induction and select the best method to be used in the future.
3. To study the larval development during the larval rearing and determine the duration taken for each growth stages and the survival rate of *H. scabra*.
4. To study the juvenile development and subsequently to determine the survival rate in the rearing tank, hapanet and growout pond.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION TO SEA CUCUMBER

The first unequivocal fossil evidence of sea cucumbers was discovered about 400 million years ago (Lambert, 1997). Sea cucumbers are members of the class Holothuroidea in the phylum Echinodermata and are sometimes referred to as holothurians or holothuroids. Sea cucumbers of various types are found throughout the tropical Pacific, as well as in other tropical and temperate seas. Over 1200 species of sea-cucumbers are known from various parts of the world but only about 70 species are being harvested (Yang et al., 2015). For example, in the seas around India nearly 200 species are known but of these only 12 species are of commercial value (James, Gandhi, Palaniswamy & Rodrigo, 1994). In Asia, a total of 125 sea cucumber species were reported from various references (Ridzwan, 1987, 1993, 2000, 2007; Ridzwan, Shukri, Fadhil, Noor Ibrahim, Kaswandi & Hawa, 1998a, Chao, 1998; Jeng, 1998; Zulfigar & Tan, 1999; Bussarawit & Thongtham, 1999; Forbes & Ilias, 1999; Flint, 2002; Zaidnuddin, 2002; Tan, 2003, 2005; Chen, 2004; Choo, 2004; Mucharin & Putchakarn, 2005; Purwati, 2005; Zulfigar, Sim & Tan, 2007; Zulfigar, Sim, Tan & Shirayama, 2008; Otero-Villanueva & Ut, 2007; Choo, 2008; Sim, Tan, Zulfigar, Wong, Chai &Ng, 2008; Sim, Tan & Zulfigar, 2009; Kamarudin, 2010) with 52 species documented as commercially important (Choo, 2004).

Morphologically, the accurate classification of sea cucumber to species level is normally based on the shapes of calcareous skin ossicle or spicule shape (e.g. table, cup, perforated plate, button, anchor and plate, wheel and basket), types of tentacle (e.g. dendritic, peltate, digitate and pinnate) and types of calcareous ring which are embedded in the skin (Lambert, 1997). Closely related species have very similar ossicles if they have recently evolved from a common ancestor (Lambert, 1997). Apart from morphological characteristics or external characters, the classification of sea cucumbers into each of the six orders is also based on habitat and behaviours, as mentioned by Kerr (2000) based on the description by Pawson (1982) and Smiley (1994).

2.2 SEA CUCUMBER OF MALAYSIA

Malaysia has been at the same time importer, consumer, producer and exporter of sea cucumber. There were various species of sea cucumber in Malaysia. Among of the species from the genus Stichopus includes *Stichopus horrens*, *S. hermanni, S. chloronotus, S. ocellata* and *S. vastus*. Holothuria species includes *Holothuria pardalis*, *H. atra*, *H. scabra*, *H. fuscogilva*, *H. edulis* and *H. leucospilta*. Most abundant species are from the genus of Holothuria and Stichopus.

Other species can be found in Malaysian waters are *Thelenota ananas* and *Thelenota anax*, from the genus Thelenota. *Colochirus quarangularis* from the genus Colochirus and *Bohadschia marmorata* from genus Bohadschia can also be found in Malaysia but in very scarce due to its habitat in the deep water. To date there are 37 species of sea cucumber identified and recorded by the Fishery Department of Malaysia (Ilias, 2010).

2.3 THE IMPORTANCE OF SEA CUCUMBER

Sea cucumbers in Malaysia are consumed as delicacy and medicine. Commonly, the sea cucumbers were processed into beche-de-mer; a sun dried or smoked sea cucumbers. *H. scabra* is a popular beche-de-mer species (Preston, 1993). The coelomic fluid of sea cucumber can be consumed as a tonic and the practice of drinking colelomic fluid from selected species of sea cucumber also occurs amongst the fishermen and the local communities in Asia; based on the belief that it could provide extra physical strength (Ridzwan, 2007).

The most commonly used body parts of the sea cucumber are the visceral organs including the gonads and intestines. These are eaten raw, cooked or fermented. 'Konawata' or preserved intestines of fermented gonads as well as 'kuchiko' (dried sea cucumber) are savoured as a favorite dish of the Japanese and Korean. Japanese use the female gonads of sea cucumber to make tea, wine and soup (Mao et. al., 2015).

In the East Malaysian states namely Sabah, sea cucumber is famous for indigenous groups such as the Sulu, Kadazan, Bajau, Sungai, Dusun and Philippines immigrants as delicacies. Often, the sea cucumber is consumed raw after the firm flesh is thinly sliced and mixed together with chilly as well as latok (*Caulerpa* sp.) (Ridzwan, 2007).

Sea cucumber is high in protein, low in fat, making it a valuable nutritional food. The body wall of sea cucumber contains polysaccharides, lipids and collagen (Bordbar et al., 2011). The active ingredients in sea cucumber are believed to allay fatigue, boost the immune system, strengthen the resistance to disease, treat injuries, prevent inflammation and provide liver and blood vessels protection (Xia & Wang, 2015).

Studies have shown that sea cucumber contains many active substances such as mucopolysaccharide acid, holothurian fucan, chondroitin sulphate, holothurin and holothurian peptides (Guan & Wang, 2009). Kariya, Mulloy, Imai, Tominaga, Kaneko & Asari (2004) had separated and purified glucosaminoglycan, a polysaccharide ideal as a lubricating fluid for joints, from *A. japanicus*. Wang, Gong, Sun, Tang, Liu and Li (2012) on the other hand had discovered nortriterpene glycoside, an antifungal from *A. japonicus* Selenka. Furthermore, new findings showed the potential of sea cucumber as an anticancer agent (Althunibat, Hashim, Taher, Mohd Daud, Ikeda & Zali., 2009).

People from Peninsular Malaysia rarely consume the sea cucumber as delicacy but they use it as medicine and regularly apply the sea cucumber extract or the coelomic fluid from the species of *S. hermanii* and *S. horrens* to traditionally heals asthma, hypertension (high blood pressure), rheumatism, skin disorders, minor wounds, burns, aches in the joints and diabetes (Ridzwan, 2007). Sea cucumber is also famous due to its power to quickly mend internal wounds especially after a surgery, caesarian birth and normal labor. Concentrated extract of the sea cucumber is said to be an effective cure to bleeding gum disease and blisters (Ridzwan, 2007).

2.4 OVER EXPLOITATION OF SEA CUCUMBER

Sea cucumbers have been exploited for a several hundred years now and the resource is insubstantial. Overexploitation of sea cucumbers has potentially serious adverse socio-economic and ecological consequences. At a community level the increasing scarcity of a high value export product would lead to increased poverty and instability in village communities (Rasolofonirina, Mara & Jangoux, 2004). From an ecological viewpoint, sea cucumbers are major components for sustaining coastal ecosystems in tropical areas, as ecosystems engineers that increase the structural complexity of the habitat and as macro-detritivores that consume various organic detritus (Coleman & William, 2002; Rasolofonirina et al., 2004).

2.4.1 Exploited Species

The major exploited holothurian species found in the south-west Pacific are listed by Preston (1993). They are *A. echinites*, *A. lecanora*, *A. mauritiana, A. miliaris, A. japonicus, H. atra, H. fuscogilva*, *H. fuscopunctata*, *H. nobilis, H. scabra*, *H. edulis, S. chloronotus*, *S. variegatus*, *T. ananas*, *T. anax*, *Bohadschia marmorata*, *B. argus* and *B. vitiensis*. All commercial sea cucumbers are members of the class Aspidochirotidae and all are relatively large species with thick body walls.

There are at least 20 species of sea cucumber in China being exploited as traditional medicine and tonic food (Chen, 2004) but the most valuable is *A. japonicus*. In Malaysia, *S. horrens* and *H. scabra* are exploited as medicine and delicacies, respectively.

2.4.2 Overview of *Holothuria scabra*

H. scabra is the most exploited sea cucumber and is the tropical species with the most potential for stock enhancement after *A. japonicus*. *H. scabra* are of high value, widely distributed and relatively easy to culture in simple systems at low cost. They live in high nutrient environments at densities of about hundreds per hectare. Generally, they have reproductive peaks in September and October, but can be induced to spawn year round. This is the most extensively processed species and also most expensive species in the world (James, 2000).

H. scabra grows to a length of 400 mm and live weight varies from 0.5 to 1.5 kg, short and stout with blunt ends and prominent wrinkles on the upper side. Upper side is grey in colour with white or yellow horizontal bands. The lower side is white in colour with a number of fine black dots. They commonly found in silty sand often near low saline areas and frequently on *Cymodocea* beds (James, 2000). It spends part of the day buried in the sand. It occurs from the intertidal region to 10 m depth. Juveniles are distributed near the shore and as they grow they migrate to deeper waters for breeding. Figure 2.1 showed the life cycle of *H. scabra*.

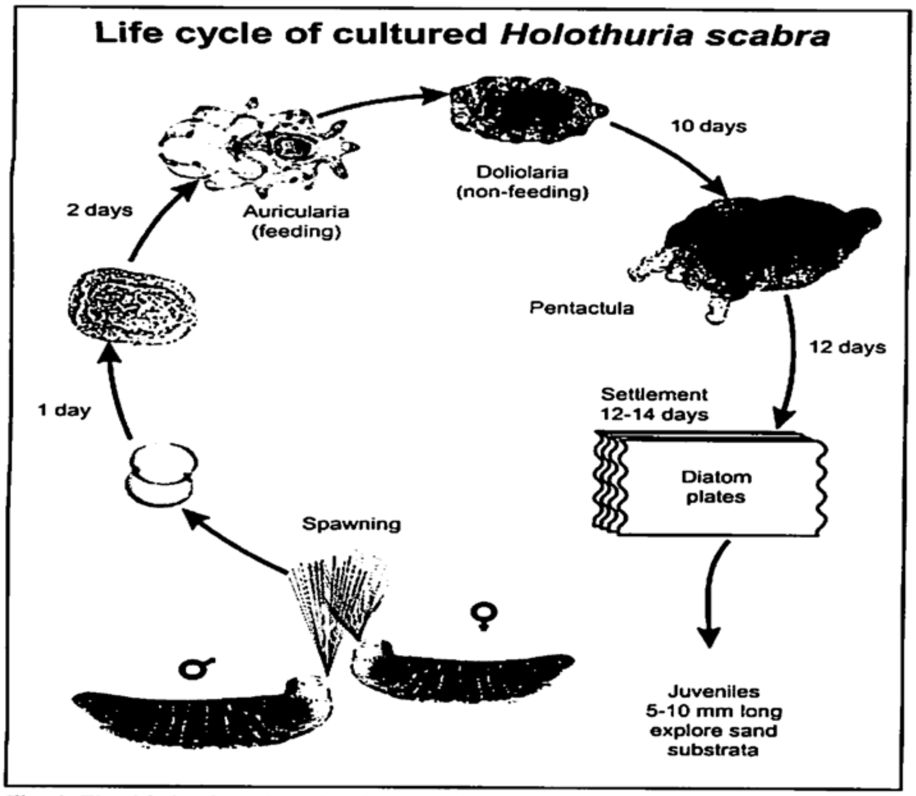

Figure 2.1 The life cycle of *Holothuria scabra*. From Battaglene, 1999.

Increases in water temperature and addition of powdered algae are effective ways of inducing spawning of *H. scabra* (Battaglene, 1999). *Chaetoceros muelleri* and *Rhodomonas salina* are two of the better microalgae for feeding the larvae (Battaglene, 1999; Lovatelli, 2004). *H. scabra* larvae are more robust and easier to rear than those of other tropical species. Larvae metamorphose into juveniles after 2 weeks at 28°C and settle on diatom-conditioned plates. They can be reared on hard substrates until they reach 20 mm in length and are then best transferred on sand substrates (Mercier, Battaglene & Hamel, 2000). Absolute daily growth rates on