UNIVERSITI PUTRA MALAYSIA

POPULATION AND REPRODUCTIVE BIOLOGY OF A COMMERCIALLY IMPORTANT SEA CUCUMBER SPECIES, *Stichopus horrens* (Selenka, 1867) IN PANGKOR ISLAND, PERAK

SYED ZULFAQAR BIN SYED MOHD KHAIR

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By

SYED ZULFAQAR BIN SYED MOHD KHAIR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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December 2018

Chair : Md. Aminur Rahman, PhD
Faculty : Institute of Bioscience

To enhance our understanding on some of the important biological aspects of commonly targeted sea cucumber species in Malaysia, population and reproduction biology of a commercially important species of tropical sea cucumber, *Stichopus horrens* (Selenka, 1867) was investigated for a period of one year in Pangkor Island, Perak, Peninsular Malaysia. In total, 641 live individuals of *S. horrens* were collected from August 2015 to July 2016. Biometrics data were measured and population parameters were estimated using length-frequency data analyzed by FAO-ICLARM Stock Assessment Tool II (FiSAT-II) software. Reproduction was monitored through macroscopic and microscopic analyses of the gonads. The mean total weight and body length of the population was 96.80 ± 2.22 g and 15.23 ± 0.19 cm respectively. The length-weight relationship of the population was found to be $W = 0.413L^{2.02}$. The growth parameter K and $L\infty$ were estimated at 0.75 $year^{-1}$ and 32.50 cm respectively. The total mortality was estimated at 2.38 $year^{-1}$, natural mortality at 1.48 $year^{-1}$ and fishing mortality at 0.90 $year^{-1}$. The level of exploitation was at 0.38 and below the optimum level of 0.50. The gonad weight and tubule diameter were significantly different between male and female, being heavier and longer in the latter. The gonad index peaked at 0.76% in September, 2015 and gradually declined to the lowest at 0.03% in May, 2016 which was also proportional to the changes in tubule diameters. Histological examination of gonads revealed that the highest proportion of mature individuals was in September, 2015 when the spawning started and prolonged up to April, 2016 with the majority spawned in October, 2015. The size-class distribution of oocyte showed the dominant distribution of large mature oocytes (80–100 µm) from October to December 2015. The size at first sexual maturity was estimated at 20 cm in length and 135 g in total weight. The mean absolute fecundity was 1,100,727 (± 148,613 SE) and significant positive relationship between fecundity and size of the individuals

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was found. The population parameters revealed that this species is a relatively fast grower with high mortality. The findings also suggested that spawning of *S. horrens* might be seasonal with major spawning occurring from October to January. The results also demonstrated that spawning took place over a long period of time and mature species can be found almost throughout the year. The species also reaches late sexual maturity and the reproductive viability increase with increasing size and also probably ages. The prolonged release of gametes and availability of matured adults throughout the year might facilitate sustainable aquaculture program of this species. The period of enhanced spawning observed will also contribute to better management of broodstocks in such program to a greater extent.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

BIOLOGI POPULASI DAN REPRODUKSI SPESIES TIMUN LAUT KOMERSIAL, *Stichopus horrens* (Selenka, 1867) DI PULAU PANGKOR, PERAK.

Oleh

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Demi menambahbaik pengetahuan tentang beberapa aspek biologi penting timun laut yang biasa menjadi tangkapan di Malaysia, suatu penyelidikan bagi menyiasat trait biologi populasi dan pembiakan spesies timun laut komersial, *Stichopus horrens* (Selenka, 1867) telah dijalankan selama satu tahun di Pulau Pangkor Perak, Semenanjung Malaysia. Sebanyak 641 sampel *S. horrens* hidup telah dikutip dari Ogos 2015 hingga Julai 2016. Data biometrik telah diukur manakala parameter populasi telah dianggarkan menggunakan data frekuensi-panjang yang dianalisa dengan perisian FAO-ICLARM Stock Assessment Tool II (FiSAT-II). Purata keseluruhan berat dan panjang badan bagi populasi ini adalah masing-masing 96.80 ± 2.22 g dan 15.23 ± 0.19 cm. Hubungan panjang-berat bagi populasi ini ialah $W = 0.413L^{2.02}$. Parameter pertumbuhan K dan $L\infty$ dianggarkan masing-masing pada 0.75 tahun^{-1} dan 32.50 cm. Kadar kematian keseluruhan ialah 2.38 tahun^{-1}, kematian semulajadi 1.48 tahun^{-1} dan kematian tangkapan 0.90 tahun^{-1}. Tahap eksploitasi bagi populasi ini ialah 0.38 iaitu di bawah paras optimum 0.50. Keputusan menunjukkan bahawa berat dan diameter gonad adalah berbeza secara signifikan antara jantan dan betina, yang mana ianya lebih berat dan lebih panjang pada betina. Indeks gonad berada pada tahap kemuncak 0.76% pada September, 2015 dan menurun secara beransur ke titik terendah 0.03% pada Mei, 2016 yang juga selari dengan corak perubahan diameter tubul. Kaedah histologi juga menunjukkan bahawa individu matang adalah terbanyak pada September, 2015. Proses mengawan bermula pada September, 2015 dan berpanjangan sehingga April, 2016 dengan majoriti mengawan pada Oktober, 2015. Kelas-saiz oosit menunjukkan taburan oosit besar dan matang, 80 - 100 µm mendominasi dari Oktober hingga Januari 2015. Saiz kematangan seksual kali pertama dianggarkan pada 20 cm panjang dan 135 g berat. Purata fekunditi absolut adalah 1,100,727 (± 148,613 SE) dan hubungan positif yang signifikan antara fekunditi dan saiz individu telah

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ditemukan. Parameter populasi menunjukkan bahawa spesies ini secara relatifnya cepat membesar dengan kadar kematian yang tinggi. Penemuan ini juga menunjukkan bahawa proses mengawan *S. horrens* mungkin bermusim dengan musim pengawanan utama dari Oktober ke Januari. Keputusan ini juga menunjukkan bahawa proses mengawan berjalan dalam jangka masa yang panjang dan individu-individu matang boleh dijumpai hampir sepanjang tahun. Spesies ini juga mempunyai kematangan seksual yang lambat dan kemampuan pembiakannya meningkat seiring dengan peningkatan saiz badan dan juga mungkin umur. Pelepasan gamet pada tempoh masa yang lama dan perolehan individu matang sepanjang tahun memungkinkan prospek suatu program akuakultur untuk spesies ini. Tempoh peningkatan pengawanan yang diperhatikan juga dapat membantu pengurusan induk yang lebih baik untuk program tersebut.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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CHAPTER 1

GENERAL INTRODUCTION

Sea cucumber (Echinodermata: Holothuroidea) or holothurian makes up a large proportion of the total marine invertebrates in the benthic ecosystems around the world. They can be found in every sea throughout the world at almost every depth. Ecologically, holothurians play an important role in nutrient and detritus recycling on the seabed (Hauksson, 1979; Uthicke, 1999; MacTavish, Stenton-Dozey, Vopel & Savage, 2012). It has been estimated that there are as much as 1400 species of holothurians existed worldwide (Kerr, 2000). Five main orders of holothurians include Apodida (Brandt, 1835), Aspidochirotida (Grube, 1840), Dendrochirotida (Grube, 1840), Elasipodida (Théel, 1882) and Molpadida (Haeckel, 1896). Among the five, Aspidochirotida, the shallow-water holothurian is the most studied order mainly due to their high commercial values in the trade market (Purcell, Samyn, & Conand, 2012). The most common and heavily exploited aspidochirotes are members in the families of Holothuriidae and Stichopodidae (Bruckner, 2006; Purcell *et al.*, 2012).

Holothurians are important trade commodity in global market. They are commonly traded as a trade unit known as bêche-de-mer (meaning sea worms) or trepang (meaning sea slugs). Bêche-de-mer in its actual term refer to smoked and dried sea cucumbers. The bêche-de-mer trade had begun since ancient times, driven primarily by Chinese demand (Toral-Granda, Lovatelli, & Vasconcellos, 2008). Today, the main markets are China, Singapore, Hong Kong and Taiwan (Ferdouse, 2004). Globalization has brought sea cucumbers into a new economic frontier. The market has been expanding due to rising demands (Purcell, Polidoro, Hamel, Gamboa, & Mercier, 2014). In 2001, the global sea cucumbers import and export have been estimated at 57 million USD and 130 million USD, respectively (Ferdouse, 2004). However, the market expansion had also caused negative effects to the global sea cucumber population. The last decades have also been the period of declining stocks for holothurians worldwide (Anderson, Flemming, Watson, & Lotze, 2011; Purcell *et al*., 2013). Many commercial fishing areas have reportedly been closed down due to the threats of severe overexploitation on several species (Anderson *et al.*, 2011). This is true for many sea cucumbers exporting countries including Malaysia, which has witnessed a major decline in its sea cucumber production year by year. The catch landings peaked at around 1,200 tons in the 80s and has been constant at around 180 tons for the past 10-15 years (Choo, 2004; 2008; see also Figure 2.2). Malaysia has also closed down one of its important sea cucumber fishery area in Langkawi Island due to near extirpation of the population of its golden sea cucumber, *Stichopus hermanni* (Baine & Choo, 1999). The Island is distinctively known for its gamat-based industry. Gamat is a local name for the sea cucumber belonging to the genus *Stichopus*, especially the species *S. hermanni* and *S. horrens*. Gamat is processed into set of

traditional medicines usually in the form of ointments (gamat oil) and tonic drinks (gamat water) prepared from boiled body walls and coelomic water of the sea cucumbers, respectively (Choo, 2004). Due to their perceived health benefits, gamat has now became a value-added ingredients into a wide range of products from toothpaste to cosmetics in Malaysia (Akamine, 2014).

Sea cucumbers, although an important marine commodity and have been commercially exploited in Malaysia for decades, are still relatively understudied especially on aspects of their population and reproductive biology. Previous researches have mostly focused on their taxonomy, distribution and diversity (Massin, Zulfigar, Tan, & Boss, 2002; Zulfigar, Sim, & Tan, 2007; Kamarudin *et al.*, 2009; Woo, Zulfigar, Norhanis, Teh, & Tan, 2010; Woo *et al.*, 2014; Kamarudin, Usup, & Hashim, 2015). Other widely researched areas on sea cucumbers in Malaysia are description and analysis of their bioactive constituents for applications in nutraceutical and pharmaceutical industries (Hawa *et al.*, 1999; Ridzwan, Leong, & Idid, 2003; Che Badariah, Asma, Mohd Nizam, & Siti, 2011; Zohdi, Zakaria, Yusof, Mustapha, & Abdullah, 2011; Forghani *et al.*, 2012; Shahrulazua, Samsudin, Iskandar, & Amran, 2013; Siddiqui *et al.*, 2013; Subramaniam, Amuthan, D'Almeida, & Arunkumar, 2013; Lukman, Nordin, & Kamarudin, 2014; Ridzwan, Hanita, Nurzafirah, Norshuhadaa, & Hanis, 2014; Forghani *et al.*, 2016). Studies on population and reproductive biology of sea cucumbers have been extensively published in Western and Pacific Island countries (Conand, 1990; Mercier & Hamel, 2009). However, to date, only a handful of researches have been published on aspects of population structure and reproductive biology of these commercially important sea cucumber species in the coastal water of Malaysia. These biological aspects are especially important in order to develop good management plans for fisheries and aquaculture. The lack of such studies will definitely hamper any effective sustainable utilization and conservation efforts of the species (Purcell, Lovatelli, Vasconcellos, & Ye, 2010). It is of no surprise that sea cucumber fishery management plan in Malaysia is lacking if not non-existent (Bruckner, 2006).

The population biology of sea cucumber particularly in relation to growth and its reproductive biology are two research gaps of sea cucumber research in Malaysia. These gaps will be reviewed within the next few paragraphs as follows:

Population biology of holothurians are studied in various ways. Most common are the description of their size structure in terms of length and weight and biometric relationships. Another important aspect of population is growth. Several methods of calculating growth such as *in situ* tagging or marking, observations in captivity, fluorescence tagging, genetic fingerprinting and size modes progression have been tried for holothurians (Conand, 1983; Wiedemeyer, 1994; Uthicke, Welch, & Benzie, 2004; Poot-Salazar, Hernández-Flores, & Ardisson, 2014). Tagging is ineffective as loss of tags is high, animals in captivity tend to cease growing and no growth rings can be properly detected in holothurians (Conand, 1983). Genetic fingerprinting involves extensive use of

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DNA analysis, which is not cheap. Methods employing temporal progression of size modes is the most promising, but several obstacles are in place. First is the problem of body size elasticity. Holothurians tend to change size and shape with different handling treatments. The variability of water content in their body also add up to the variability in the measurements. As such, determination of proper size is difficult. However, some authors have suggested several modifications that can be made to the biometric components to account for this problem (Yamana & Hamano, 2006; Poot-Salazar *et al.*, 2014). Secondly, there is another problem which is the lack of clearly defined mode for sizes (Conand, 1981; Uthicke *et al.*, 2004). These problems can be encountered by using bigger sample size and lack of defined size modes does not necessarily applied to all holothurian population. In addition, use of modeling software such as FiSAT (FAO-ICLARM Stock Assessment Tool) and LFDA (Length Frequency Data Analysis) have made growth estimation using monthly size or length data more objective and robust. These tools also allow for other population parameters such as recruitment and mortality to be estimated. To date, there were only two researches that try to investigate population biology of sea cucumber population in Malaysia (Rahmad & Zulfigar, 1993; Nuraini, 1995). This is inadequate considering the importance of sea cucumber especially as a valuable commodity in this country.

Earlier studies on the reproduction of holothurians have shown that most holothurians breed at a distinctive time of the year with only some few exceptions (Hyman, 1955; Boolotian, 1966), however those were mostly concentrated on temperate regions. Since Bakus (1973) reviewed the limited accounts on reproductive aspects of tropical holothurians, reproductive studies on tropical species has since been tremendously grown (Smiley, McEuen, Chaffee, & Krishnan, 1991; Mercier & Hamel, 2009). Despite the increasing literatures, some questions still remain. One such question is the nature of spawning pattern in tropical holothurians - is it aperiodic or seasonal? Some authors have observed that most tropical species especially the ones closer to equator have an aperiodic spawning pattern (Ong Che & Gomez, 1985; Toral-Granda & Martínez, 2007). However, some of the equatorial species has shown to have an annual cycle (Ong Che, 1990). Mercier and Hamel (2009) stated that spawning periodicities can easily be masked for example with an inadequate sampling. Some species with supposedly aperiodic spawning were observed to exhibit periodicity with longer observation (Mercier, Ycaza, & Hamel, 2007). Furthermore, aperiodicity at the population level does not necessarily means the same at the individual level (Mercier & Hamel, 2009). Malaysia is located near to the equator which is home to diverse sea cucumber species (Kamarudin *et al.*, 2015). However, there is scarcity of data and very limited information on the reproductive biology of these holothurians. Previous studies are mostly not very comprehensive typically with low sample sizes and short study durations (Sallehudin, 1992; Yang, 1997; Rodzi, 2001). Thus, there is a pressing need to get some of the biological data, especially on commonly-exploited species in Malaysia to add into our knowledge on reproductive biology of tropical holothurian, particularly those in equatorial region.

The knowledge gaps on the study of sea cucumbers in Malaysia presented here are worth exploring, especially in aiding effort to promote conservation, sustainable fishery, and aquaculture effort of commercially important sea cucumber species in Malaysia. One of such species is *Stichopus horrens* also known as the golden gamat. This species is one of the key target species and has currently been exploited to be utilized in the production of gamat-based products in Malaysia. However, the knowledge on biology and ecology of *S. horrens* is very scarce both in Malaysia and in the world (Conand, Purcell, & Gamboa, 2013).

Based on these gaps, the research has been conducted on the population of *S. horrens* in Pangkor Island, Perak in order to delineate aspects of their population and reproductive biology. The objectives of the research are as follows:

i) To determine the size structure, biometric relationships, growth, recruitment, mortality and exploitation of the population.

ii) To determine some aspects of the reproduction biology of the population including the characteristic of the gonads at different maturity and gametogenic stages, size at first sexual maturity, fecundity, spawning pattern and exogenous cues for spawning.

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Syed Zulfaqar was born on 18 April 1992 in Kuala Lumpur, Malaysia. He started his early education in Sekolah Rendah Ibn Kathir in Kampung Changkat, Gombak. From there, he went for his secondary schooling in Sekolah Menengah Ibn Khaldun, Klang and Maahad Attarbiyyah Al-Islamiyyah (MATRI), Perlis. He then received a JPA sponsorship to further his tertiary study in Canada. In June 2015, he graduated with a bachelor degree in molecular biology from University of Toronto.

During his undergraduate years, he had interned at the Ontario Ministry of the Environment and Climate Change in Sportfish Contaminant Monitoring Program unit, where he researched on the relationship between specific polychlorinated biphenyl (PCB) congeners with total PCB content in fishes. He was also very captivated with a course on field ecology that he took as an elective during those years. These experiences are perhaps instrumental in program selection of his master's degree in the field of marine ecology and biodiversity here in UPM.

He was blessed with a loving wife, a beautiful daughter and a son. He currently resides in Shah Alam, Selangor.

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LIST OF PUBLICATIONS

Paper publications

Zulfaqar, S., Rahman, M.A., Yusoff, M.F. and Arshad, A. Population Biology of a Commercially Important Sea Cucumber Species (Echinodermata: Holothuroidea), *Stichopus horrens* in Pangkor Island, Perak. (draft)

Zulfaqar, S., Rahman, M.A., Yusoff, M.F. and Arshad, A. Some Aspects of Reproductive Biology of a Commercially Important Sea Cucumber Species (Echinodermata: Holothuroidea), *Stichopus horrens* in Pangkor Island, Perak. (draft)

Zulfaqar, S., Rahman, M. A. and Yusoff, M.F. (2016). Trends, prospects and utilizations of sea cucumber fisheries in Malaysia. *International Journal of Advances in Agricultural & Environmental Engineering* (IJAAEE), 3 (1). pp. 114-116.

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Zulfaqar, S., Rahman, M.A., Yusoff, M.F. and Arshad, A. (2017). Reproductive Biology of a Commercially Important Sea Cucumber, *Stichopus horrens* in Malaysia: Important Insights for Aquaculture Development*. Asian-Pacific Aquaculture 2017 (APA17)* Kuala Lumpur, Malaysia. July 24 - 27, 2017

Poster presentation

Rahman, M.A., **Zulfaqar, S.**, Yusoff, M.F. and Arshad, A. (2016). Trends, Prospects and Utilizations of Sea Cucumber Fisheries in Malaysia. *Pameran Rekacipta, Penyelidikan dan Inovasi 2016 (PRPI16)*, Universiti Putra Malaysia. Nov 15 – 16, 2016

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