

**THE EFFECTS OF PHYSICAL STRESSORS AND
NEUROTRANSMITTERS ON THE SPAWNING
ACTIVITY OF THE SEA CUCUMBER, *Stichopus
fusiformiossa***

CHEONG KAH MEI

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by

CHEONG KAH MEI

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LIST OF SYMBOLS

μm	Micrometer
%	Percent
$^{\circ}\text{C}$	Degree Celsius
L	Litre
mL	Millilitre
g	Gram
kg	Kilogram
\pm	Plus or minus
n	Sample size
$\text{\textcircled{M}}$	Male
$\text{\textcircled{F}}$	Female

LIST OF ABBREVIATIONS

1-MA	1-Methyladenine
5-HT	Serotonin
GI	Gonad index
GV	Germinal vesicle
GVBD	Germinal vesicles break down
H&E	Haematoxylin and eosin
SE	Standard error
t	Metric tonne
min	Minute
hrs	Hours
d	Day
USD	United States dollar
ppt	Represent salinity in parts per thousand

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**KESAN TEKANAN FIZIKAL DAN PENGHANTAR NEURON TERHADAP
AKTIVITI PEMBENIHAN GAMAT, *Stichopus fusiformiossa***

ABSTRAK

Stichopus fusiformiossa, sejenis gamat daripada keluarga Stichopodidae, telah lama wujud dalam perubatan tradisional Malaysia dan kini semakin diguna dalam industri farmaseutikal, nutraseutikal, kosmetik, serta produk makanan. Namun, eksploitasi berlebihan tanpa langkah pemuliharaan yang sewajarnya, meletakkan spesies gamat ini dalam terancam. Untuk menangani isu ini, meningkatkan kaedah pembiakan, khususnya pembenihan buatan, adalah penting untuk akuakultur yang mampan. Oleh itu, kajian ini bertujuan untuk membangunkan protokol pembenihan untuk *S. fusiformiossa* dengan menilai potensi penghantar neuron dalam mengawal pelepasan gamet dan persenyawaan. Kajian ini merupakan percubaan pertama yang didokumentasikan untuk mendorong pembenihan dalam *S. fusiformiossa* menggunakan kaedah fizikal: kejutan haba, kejutan sejuk, pengeringan, dan rangsangan mikroalga dan suntikan penghantar neuron: serotonin (5-HT), 1-metiladenina (1-MA), epinefrina, dan norepinefrina dalam kurungan. Gamat tersebut diperhatikan selama lapan jam selepas induksi. Analisis histologi gonad dijalankan secara rawak untuk memberikan gambaran mengenai peringkat perkembangan pembiakan. Ujian pemecahan vesikel germinal (GVBD) secara *in vitro* telah dijalankan ke atas oosit *S. fusiformiossa*, menggunakan 5-HT dan 1-MA pada dua kepekatan (1×10^{-1} M dan 1×10^{-3} M) selama tiga tempoh inkubasi (60, 120, dan 180 minit). Aktiviti pra-bertelur seperti mengangkat kepala, mengayunkan bahagian hadapan (samping ke sisi, ke belakang dan ke hadapan, kepala berputar/berpusing), dan keterlihatan ketara gonopore, ditunjukkan oleh *S. fusiformiossa* sepanjang tempoh

lapan jam selepas induksi. Semua penghantar neuron berkesan mencetuskan aktiviti pra-bertelur, dengan gamat yang dirawat dengan 1-MA menunjukkan aktiviti yang paling tinggi sepanjang tempoh pemerhatian. Walaupun gamat menampilkan aktiviti pra-bertelur, rawatan fizikal dan suntikan penghantar neuron yang diberikan tidak berjaya menyebabkan *S. fusiformiossa* bertelur. Namun demikian, penghantar neuron menunjukkan potensi dalam merangsang kematangan oosit, dengan 5-HT pada kepekatan 10^{-1} M menghasilkan kadar pemecahan sel folikel yang signifikan (55%) 1-metiladenina selepas dua jam inkubasi. Tiada penghantar neuron yang dapat menginduksi GVBD, yang menghalang oosit daripada menjadi boleh disenyawakan. Penemuan ini mencadangkan bahawa walaupun induksi penetasan masih merupakan cabaran, penghantar neuron mungkin dapat membantu dalam proses persenyawaan buatan. Kajian lanjut adalah penting untuk memperhalusi kaedah ini bagi akuakultur gamat yang mampan.

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ABSTRACT

Stichopus fusiformiossa a sea cucumber species from the Stichopodidae family, known as *gamat* have been integral to Malaysian traditional medicine and is now increasingly valued in pharmaceuticals, nutraceuticals, cosmetics, and food products. However, excessive harvesting and insufficient conservation measures have endangered this sea cucumber species. To address this issue, enhancing breeding methods, specifically artificial spawning, is essential for sustainable aquaculture. Therefore, this research aims to develop a spawning protocol for *S. fusiformiossa* by investigating the potential of neurotransmitters to regulate gamete release and fertilisation. This research represents the first attempt to induce spawning in *S. fusiformiossa* using physical methods: heat shock, cold shock, desiccation, and microalgae stimulation and neurotransmitter injections: serotonin (5-HT), 1-methyladenine (1-MA), epinephrine, and norepinephrine in captivity. The sea cucumbers were observed for eight hours post-induction. Histological analysis of the gonads was conducted randomly and provided insights into reproductive development stages. An *in-vitro* germinal vesicle breakdown (GVBD) assay was conducted on *S. fusiformiossa* oocytes, using 5-HT and 1-MA at two concentrations (1×10^{-1} M and 1×10^{-3} M) over three incubation durations (60, 120, and 180 minutes). Pre-spawning behaviours, such as raising their head, swaying the anterior (side to side, back and forth, head spinning/circling), and prominent visibility of the gonopore, were displayed by *S. fusiformiossa* for eight hours post-induction. All neurotransmitters

effectively triggered pre-spawning behaviours, with the sea cucumbers treated with 1-MA displaying the highest activity during the eight-hour observation period. Despite exhibiting pre-spawning behaviours, none of the treatments successfully induced spawning in *S. fusiformiessa*. However, neurotransmitters showed promise in promoting oocyte maturation, with 5-HT at 10^{-1} M resulting in a significant rate (55%) of follicle cell breakdown compared to 1-MA at 2 hours post-incubation. Neither neurotransmitter was able to induce GVBD, which prevented the oocytes from becoming fertilisable. These findings suggest that while inducing spawning remains a challenge, neurotransmitters may assist in artificial fertilisation. Further research is essential to refine these methods for sustainable sea cucumber aquaculture.

CHAPTER 1

INTRODUCTION

1.1 Research background

Sea cucumbers are marine invertebrates classified as echinoderms, similar to sea urchins and sea stars. They are characterised by their flexible, spiny skins and cylindrical bodies, moving sluggishly on the ocean floor and feeding using their feeding tentacles (Taiyeb-Ali et al., 2003). Tropical sea cucumbers of the family Stichopodidae are commercially significant, with high economic value, and are widely harvested in the western and central Pacific regions due to their edibility and medicinal properties. These species are primarily nocturnal, spending the day sheltering in seagrass beds, rock crevices, or reef habitats (Hartati et al., 2021).

In Malaysia, *Stichopus horrens* have been used as traditional medicine for centuries (Bakar et al., 2020). *S. horrens* and *Stichopus hermanni* often known as *gamat emas* or golden sea cucumber, are commercially processed into *gamat* oil (lipid extracts) and *gamat* water (body fluid extracts) (Kamarudin and Rehan, 2015; Kamarudin et al., 2010; 2016; 2017; Baine and Choo, 1999). The people of Langkawi Island believe that *gamat* water extract is a miraculous treatment for wounds, inflammation, sores, asthma, and stomach ulcers (Taiyeb-Ali et al., 2003). Additionally, communities around the east and west coasts of Peninsular Malaysia utilise the ointment (*gamat* oil) to alleviate joint and back discomfort (Taiyeb-Ali et al., 2003).

Sea cucumbers from the Stichopodidae and Holothuriidae families are commonly processed into products such as *bêche-de-mer* or *trepan* (dry tunics), as well as frozen, pickled, and canned forms (Kamarudin et al., 2016; 2017; 2018). Due

to their valuable medicinal properties, including tissue regeneration, wound healing, blood nourishment, kidney tonification, gastroprotection, and dermatological benefits, they are widely utilised in the nutraceutical, pharmaceutical, and cosmetic industries to meet global demand (Kamarudin et al., 2016; Rioja et al., 2020). Increasing seafood consumption, trade, and overexploitation have raised global conservation concerns (Zulfaqar et al., 2016). As a high-protein, low-lipid marine resource, sea cucumbers provide nutritional benefits while also playing a vital ecological role. As deposit feeders, they contribute to sediment bioturbation, nutrient cycling, and the regulation of materials and energy flow in marine ecosystems (Rakaj et al., 2019, Purcell et al., 2016).

Aquaculture, or artificial breeding, is a key strategy for restocking depleted sea cucumber populations and ensuring their sustainable utilisation. Continuous seed production and larval culture require healthy, mature gametes from broodstock, for the success in artificial breeding (Abidin et al., 2019). Additionally, effective artificial spawning techniques that minimise stress are crucial for breeding success in captivity (Ajayi and Amedu, 2020). Currently, Malaysia Malaysia lacks commercial aquaculture farms cultivating stichopodid sea cucumbers. Thus, this research aims to develop a reliable artificial spawning induction protocol for *S. fusiformiossa*, utilising neurotransmitters to enhance gamete availability for aquaculture. Potentially, chemically induced methods with the utilisation of neurotransmitters may improve spawning success in *S. fusiformiossa* while reducing exposure to stressors. The output of this study could support sustainable farming by scaling up the production of *S. fusiformiossa* in Malaysia to meet the demands of the pharmaceutical, nutraceutical, and cosmetic industries. Furthermore, findings may have broader applications for other tropical sea cucumber species

1.2 Problem statement

The rising global demand for valuable sea cucumbers has led to the overharvesting of wild populations, resulting in several species being classified as threatened. Sea cucumbers are susceptible to overexploitation because of their slow recruitment rates, late maturation, and reproductive success that depends on population density (Al-Rashdi and Claereboudt, 2018; Acosta et al., 2021). Aquaculture could help replenish wild populations and provide a sustainable way to harvest sea cucumbers. However, successful breeding in captivity remains challenge. The main bottleneck in sea cucumber aquaculture is the difficulty in inducing consistent and controllable spawning, as well as achieving high fertilisation success in captivity (Chaiyamoorn et al., 2018; Nontunha et al., 2020; Nocillado et al., 2022).

Artificial spawning induction has the potential to overcome these challenges, yet it remains underdeveloped for *S. fusiformiossa*. Understanding the neurohormonal mechanisms that regulate gamete release is essential for improving spawning efficiency and success (Chaiyamoorn et al., 2018). Neurotransmitters play a critical role in controlling gamete release in many marine species, but their effect on *S. fusiformiossa* has not been fully explored.

This study aims to fill this gap by developing a protocol for the artificial induction of spawning in *S. fusiformissa*, utilising neurotransmitters to control gamete release and fertilisation. Additionally, the study will assess the gonadotropic activity of neurotransmitters on *S. fusiformiossa* oocytes *in vitro*. By addressing these objectives, this study will contribute to more reliable and sustainable sea cucumber aquaculture, reducing pressure on wild populations and supporting the long-term viability of this valuable species.

1.3 Objectives in this study

The objectives in this study are:

- i. To implement and develop a protocol for artificial spawning induction in *S. fusiformiossa* with the utilisation of neurotransmitters for controllable gamete release and fertilisation.
- ii. To assess the gonadotropic activity of neurotransmitters on *S. fusiformiossa* oocytes *in-vitro*.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Sea cucumbers are benthic macrofauna with a cylindrical body and pentameric radial symmetry. They have an elongated, vermiform, soft-bodied structure with rough, leathery, and prickly skin (Pangestuti and Arifin, 2018). One of their most notable features is their ability to regenerate all internal organs lost during evisceration—a process triggered by physical or chemical stress (Manan et al., 2016). Additionally, some species can undergo asexual reproduction by splitting into two, with each half regenerating into a new individual (Manan et al., 2016; Hartati et al., 2019; Ahmed and Ali, 2020).

Sea cucumbers are distributed globally, inhabiting diverse environments from tidal areas to the deep ocean. They typically reside on rocky or hard surfaces, in soft sediments, or as epizoites on aquatic plants. Known by various cultural names, namely *bêche-de-mer* (French), *trepan* (Indonesian), *namako* (Japanese), *balatan* (Filipino), and *gamat* (Malay) (Barzkar et al., 2017). Their feeding habits and ecological roles make them essential to marine ecosystems. As detritivores and deposit feeders, they consume organic matter, aiding in sediment bioturbation, mineralisation, nutrient recycling, algae growth, carbonate level regulation, energy transfer within the food chain, and biodiversity enhancement through symbiosis with other marine organisms (Conand, 2018; Balogh et al., 2019; Venâncio et al., 2022; Cheng et al., 2021).

Stichopus sp., a prominent species in tropical shallow waters, is a crucial fishery commodity (Woo et al., 2015). Stichopodids which provide useful compounds for biotechnology and medicine, are being overharvested to meet consumer demand,

despite their high commercial value (Kamarudin et al., 2017). Overharvesting negatively impacts communities, depletes resources, and degrades ecosystems. Thus, sustainable management practices and regulations are crucial for the long-term sustainability of valuable resources.

2.2 Taxonomy of *S. fusiformiossa*

Sea cucumbers are members of the class Holothuroidea of the phylum Echinodermata. Approximately 7,000 echinoderm species exist worldwide, classified into five groups: Crinoidea (crinoids), Asteroidea (asteroids), Ophiuroidea (ophiuroids), Echinoidea (echinoids), and Holothuroidea (holothuroids) (Dupont et al., 2010). Holothuroid was named by the Greek philosopher Aristotle (from the Greek words "holos," which means whole, and "thurios," which means rushing) (Bordbar et al., 2011). Holothurians differ from other echinoderms by having an anterior calcareous ring that connects the oesophagus and buccal tentacles, which assist in feeding. Additionally, holothurians have a unique external skeleton composed of tiny ossicles and a robust haemal system. They are categorised based on calcareous ring morphology, ossicle shape and arrangement, the number and shape of buccal tentacles extending from the mouth, and the presence of respiratory trees (Junus et al., 2018). There are seven orders in the class Holothuroidea: Dendrochirotida, Synallactida, Molpadida, Persiculida, Holothuriida, Elasipodida, and Apodida (Miller et al., 2017; Sulardiono et al., 2022).

Macroscopic studies of internal organs and external morphology are commonly used to identify species in sea cucumbers, with ossicle examination being the most crucial trait. Ossicles are tiny, calcified fragments in the sea cucumber skeleton. However, ossicle inspection is a laborious and highly skilled method for species identification. DNA barcoding is a molecular taxonomy technique that uses mitochondrial markers, specifically cytochrome oxidase subunit 1, or CO1 markers, to quickly and accurately identify closely related sea cucumber species (Madduppa et al., 2017).

A total of eleven species from the Family Stichopodidae were identified, belonging to two genera, *Stichopus* and *Thelenota*. The species include *S. horrens*, *Stichopus pseudohorrens*, *Stichopus naso*, *Stichopus vastus*, *Stichopus chloronatus*, *S. herrmanni*, *Stichopus monotuberculatus*, *Stichopus ocellatus*, *Stichopus quadrifasciatus*, *Thelenota anax*, and *Thelenota ananas* (Hartati et al., 2021). *S. naso*, *S. monotuberculatus*, *S. horrens*, *S. herrmanni*, *S. quadrifasciatus*, and *S. fusiformiossa* exhibit interspecific and intraspecific similarities, often misidentified due to their similar morphological features (Massin, 2007; Byrne et al., 2010; Wen et al., 2010; Cheng et al., 2021).

S. fusiformiossa from the Straits of Malacca belongs to the Echinodermata phylum, class Holothuroidea, and family Stichopodidae. *S. fusiformiossa* shares a striking resemblance with *S. horrens*. Both of them share morphologically similar body colours, which range from beige to grey-brown with sporadic black and grey patches on the dorsal part. A distinctive feature of *S. fusiformiossa* is the presence of fusiform and spindle-like ossicles in the tentacles, while *S. horrens* has tack-like tables ossicles in its dorsal papillae (Woo et al., 2015). *S. naso* is characterised by diagnostically large C-ossicles (Byrne et al., 2010).

Based on ossicle features, the sea cucumber employed in this study is therefore identified as *S. fusiformiossa*, as described by Woo et al. (2015).



Figure 2.1: *S. fusiformiossa*, (A) dorsal view and (B) ventral view

2.3 Current status of sea cucumber fisheries and stocks: global, Asia-Pacific, and Malaysia perspectives

In the Pacific region, offshore fisheries production grew steadily from 1999 to 2014, whereas coastal fisheries production remained stable despite increased fishing efforts in coastal areas across most countries. Offshore fishing, primarily undertaken by large industrial vessels using purse-seine, longline, and pole-and-line gear to target tuna, produces much larger catches than coastal fisheries. A 2016 SPC study revealed that coastal commercial production had the highest unit value (USD 4.00 per kg) compared to coastal subsistence (USD 2.10 per kg), offshore locally based (USD 1.76 per kg), and offshore foreign-based production (USD 1.57 per kg), mainly due to the high market prices for invertebrate species like *bêche-de-mer* and aquarium fish (Gillett and Tauati, 2018).

Sea cucumber fishing is predominantly a coastal subsistence or artisanal activity, typically harvested by hand through free-diving, gleaning, or using SCUBA or hookah gear (Gillett and Tauati, 2018; Purcell et al., 2023). Globally, 66% of sea cucumber fisheries are small-scale operations focused on export, while 27% operate illegally despite national moratoria, 4% are aimed at subsistence and local consumption, and 3% involve both industrial and small-scale fishing activities (Purcell et al., 2011). In Malaysia, the sea cucumber fishery follows a similar artisanal pattern, with fishing efforts concentrated in Langkawi, Pangkor, Pulau Payar, and Pulau Besar in Peninsular Malaysia, and in Semporna, Sandakan, Kudat, Kota Marudu, Kota Belud, and Kota Kinabalu in Sabah (Baine and Choo, 1999; Choo, 2008). However, since the mid-1990s, there have been no landings from Kota Marudu and Kota Belud, suggesting overexploitation and possible depletion of sea cucumber stocks in these areas (Choo, 2008). In Pangkor, *S. horrens* is hand-picked during low spring tides,

which typically last 5 days (Baine and Choo, 1999; Choo, 2008). Since the mid-1990s in Sabah, 70–80% of sea cucumbers have been harvested through hand-picking or free diving, whereas earlier data indicated that over 90% were bycatch from trawling (Choo, 2008). Fishermen in the region primarily target species such as *T. ananas*, *Holothuria nobilis*, *H. fuscogilva*, and *Holothuria scabra*, with most of the catch being sent to the export market (Baine and Choo, 1999). In the 1990s, overfishing in Langkawi, coupled with high market demand from Thailand, contributed to a decline in the population of *Stichopus* spp. (Baine and Choo, 1999).

With approximately 1,716 species of sea cucumbers worldwide, the Asia-Pacific region hosts the highest diversity (Pangestuti and Arifin, 2018). This region also accounts for the greatest exploitation, with 52 species in Asia and 36 species in the Pacific (Toral-Granda et al., 2008). Indonesia, situated in the Coral Triangle—the global centre of marine biodiversity—is recognised as the world’s oldest and largest supplier of sea cucumbers (Pangestuti and Arifin, 2018; Ardiansyah et al., 2021). At least 26 out of 350 sea cucumber species from Indonesian waters are valuable (Pangestuti and Arifin, 2018). Similarly, Malaysia, which also lies within the Coral Triangle, benefits from rich marine biodiversity and serves as a key exporter of sea cucumber products (Zulfaqar et al., 2016). Malaysia's marine heritage consists over 80 species sea cucumbers, with *Stichopus* spp. being harvested for medicinal purposes (Kamarudin et al., 2015; Kamarudin et al., 2010; Zulfaqar et al., 2016; Manan et al., 2016). Sea cucumbers commonly exploited are from the families Holothuriidae and Stichopodidae, specifically the genus *Actinopyga*, *Bohadschia*, *Stichopus* and *Holothuria* (Choo, 2008).

Global wild sea cucumber populations are being severely over-exploited and depleted, particularly in the Indo-Pacific, with about 20% of fisheries depleted, 38%

over-exploited, 14% were fully exploited, and 27% were underexploited or moderately exploited (Purcell et al., 2011). The growing consumption of sea cucumbers in the Asian region, along with extensive harvesting and trade in over 90 countries, is further exacerbating the pressure on these populations (Purcell et al., 2023). Conand (2018) noted a significant increase in global tropical fisheries for holothurians between 1986 and 2014, highlighting overexploitation in traditional fishing nations across the West Pacific and Indian Ocean. Between 1986 and 1996, total exploitation was 10,489 t per year across 19 countries. However, between 2009 and 2014, this number sharply rose to 16,026 t per year from 30 nations. Currently, Canada is the largest harvester of wild sea cucumbers, sourcing 15% from the Pacific coast and 85% from the Atlantic coast (Mercier et al., 2025). The Central West Pacific is the leading exporter, with an annual average of 6,675 t, followed by the East Indian Ocean (4,179 t), the West Central Atlantic (2,667 t), the Indian Ocean West (1,555 t), the Pacific Central East (935 t), and the Pacific Southeast (15 t) (Mercier et al., 2025).

High fishing pressure has affected most commercial stocks, with year-round, intense, and widespread fishing, as well as international traders using local agents to purchase sea cucumbers from fishers, even from remote islands (Friedman et al., 2011). Aspidochirotidae sea cucumbers from the Holothuriidae and Stichopodidae families, are suffering from overfishing due to unsustainable fisheries (Friedman et al., 2011). In Tonga, high-value sea cucumber stocks: *Holothuria fuscogilva*, *Holothuria whitmaei*, and *T. ananas* declined significantly between 1990 and 1996, with densities in 1996 being 51–85% lower than in 1990, leading to a ban on exports due to overfishing. Similarly, in Fiji, sea cucumber densities for species such as *H. fuscogilva*, *H. whitmaei*, and *H. scabra* dropped between 2003 and 2009, with densities in 2009 being 70–93% lower than in 2003 (Friedman et al., 2011). *Apostichopus japonicus*,

Isostichopus fuscus, *T. ananas* are classified in IUCN status as Endangered, indicating they are at high risk of extinction. Meanwhile, *Apostichopus parvimensis* and *S. herrmanni* are classified in IUCN status as Vulnerable (Mercier et al., 2025).

The overexploitation of sea cucumbers in Malaysia has led to a marked decline in their populations. Langkawi Island, historically recognised as the 'City of Gamat,' has experienced a significant reduction in *S. herrmanni* (Zulfaqar et al., 2016). A similar trend has been observed in Pangkor Island where in 1994, divers could collect a large bucket of *gamat* within an hour. In contrast, by 2004, the population had declined to the extent that even obtaining three large sea cucumbers had become a challenge (Choo, 2008). FAO and Department of Fisheries, Sabah statistics indicate a nearly tenfold decline in sea cucumber landings, from approximately 1,000 t (wet weight) in the 1990 to 100 t (wet weight) in the 2000 (Choo, 2008).

The depletion of higher-value species due to overfishing has caused fisheries to shift their focus to targeting low- and medium-value species. For example, after the depletion and collapse of *I. fuscus*, *S. horrens* became the target in the Galápagos Islands (Hearn and Pinillos, 2006).

Global sea cucumber aquaculture production rose from 2011, peaking in 2017, and after a decline in 2018 and 2019, rebounded to approximately 225,000 t (wet weight) in 2021, with China being the world's largest producer of farmed sea cucumbers (Mercier et al., 2025). Aquaculture plays a crucial role in meeting market demand while supporting wild population recovery by replenishing stocks with hatchery-raised juveniles, offering a sustainable alternative to traditional fisheries (Hu et al., 2013). To date, artificial propagation has been successfully implemented in commercially valuable holothurian species, including *A. japonicus*, *H. scabra*,

Holothuria leucospilota, *S. horrens*, and *S. monotuberculatus*, (Agudo, 2006; Hu et al., 2013; Kumara and Dissanayake, 2015; Vaitilingon et al., 2016; Huang et al., 2018; Cheng et al., 2021; Lin and Nan, 2022).

2.4 Commercial value of sea cucumber globally, in the Asia-Pacific, and in Malaysia

Sea cucumber harvesting and trade in the Pacific islands (India, Indonesia, and the Philippines) began in the 16th century, driven by demand from China for consumption (Akamine, 2004; Akamine, 2014). By the late 17th century, Japan and Southeast Asian countries joined the trade, harvesting sea cucumbers for export to China, and in the 18th century, South Pacific islands began processing dried sea cucumbers (Akamine, 2004; Akamine, 2014).

In the Pacific region, around 30 species of sea cucumbers are actively harvested for export, with the most economically valuable species including *H. fuscogilva*, *Holothuria lessoni*, *H. scabra*, *H. whitmaei*, and *T. ananas* (Purcell, 2014). Most sea cucumbers are processed into dried *bêche-de-mer* and exported to Asian seafood markets, driven by their high market value. Hong Kong (China), Singapore, and Taiwan (Province of China) are the main sea cucumber markets and re-exporting centres (Conand, 2004). Hong Kong is the world's largest market and importer of *bêche-de-mer* from the Pacific region, receiving 82% from Tonga, 75% from Kiribati, 100% from Fiji, and 80% from New Caledonia (Purcell, 2014). In 2021, the top five sea cucumber exporters by volume were Canada, Russia, Hong Kong, Malaysia, and Taiwan, while the top importers were Saudi Arabia, mainland China, Hong Kong, Malaysia, and the United States. By value, the leading exporters were Japan, Canada, Hong Kong, South Korea, and Nicaragua, and the top importers were Hong Kong, mainland China, Taiwan, the United States, and Singapore. China remains the largest end user of sea cucumber products (Mercier et al., 2025).

Retail market surveys conducted in Hong Kong and Guangzhou, China, between 2011 and 2016 revealed that the average price of high-value sea cucumber

species ranged from USD 369 to USD 1,898 per kg, with an annual growth rate of approximately 2.4% (Purcell, 2014; Purcell et al., 2018; Govan, 2018). According to a market study in China, the most valuable *beche-de-mer* species are the largest sandfish (*H. scabra*), valued at US\$ 1,668 per kg in Hong Kong with an average price of US\$ 303 per kg, and the golden sandfish (*H. lessoni*), which has an average price of US\$ 385 per kg (Purcell, 2014). The largest increases in price relative to export price were seen in *H. scabra* (4.0 times), *H. lessoni* (3.8 times), *S. herrmanni* (3.7 times), and *Actinopyga lecanora* (3.6 times) (Purcell, 2014).

In the Guangzhou, China market, the average selling price of *S. horrens beche-de-mer* surged from USD 69 per kg in 2011 to USD 119 per kg in 2016 (Govan, 2018). A report on illegal fishing of *gamat* in Pasir Puteh, Kelantan, revealed that high prices and demand in Vietnam have prompted Thailand's fishermen to invade Malaysian waters, resulting in marine habitat destruction. In contrast to Malaysia, where *gamat* is marketed for between RM60 and RM500 per kg, *gamat* is more expensive in Vietnam (RM1,000 per kg) (Rahim, 2021).

2.5 Importance and uses of sea cucumbers: Global, Asia-Pacific, and Malaysia

Chinese have consumed sea cucumber as a tonic and a traditional medicine for centuries since the Ming Dynasty (1368-1644 BC) (Chen, 2004). Also, sea cucumbers are a significant dietary component in other Southeast Asian populations, including those of Singapore, Vietnam, Korea, Malaysia, and Japan (Purcell, 2014). In traditional Chinese medicine, sea cucumbers have warming properties are believed to nourish blood; tonifying kidney by enhance *jing* (vital essence) and *qi* (kidney energy); support reproductive health; and treat conditions such as weakness, impotence, age-related debility, constipation due to intestinal dryness, and frequent urination (Chen, 2004). Due to medicinal properties of the sea cucumber, Chinese named sea cucumber as “*haishen*”, meaning “ginseng of the sea” (Chen, 2004).

Sea cucumbers have been validated through scientific and ethnopharmacological research to contain bioactive compounds, including triterpene glycosides (saponins), angiotensin-converting enzyme (ACE) inhibitory peptides, chondroitin sulphate, lectins, heparin, cerebroside, gangliosides, sterols, and omega-6 and omega-3 fatty acids that are highly valued in the food, nutraceutical and pharmaceutical sectors (Bordbar et al., 2011; Forghani et al., 2012; Zhao et al., 2018). They are renowned for their neuroprotective, anti-tumour, anti-coagulant, anti-microbial, anti-oxidant, anti-hyperlipidemic, anti-inflammation and anti-osteoclastogenesis properties, attributed to their rich protein content, low lipid levels, and abundant collagen, gelatin, polysaccharides (Pangestuti and Arifin, 2018; Oh et al., 2017). Extracts from sea cucumbers have demonstrated anti-inflammatory effects, ease post-surgical pain, accelerate wound healing, hypertension (Ridzwan et al., 2003; Forghani et al., 2012; Wen et al., 2018). Also, they are a source of chondroitin sulphate

(glycosaminoglycan) to reduce arthritic pain for treating rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Chen, 2004). Thus, the growing market demand for sea cucumber underscores its medical efficacy, driving advancements in both farming and fishing.

In the global market, the intestine and gonads of *S. naso* are traditionally consumed as food (Hartati et al., 2021). *S. horrens* is a valuable marine resource and a source of livelihood for the coastal communities in many countries like Indonesia, the Philippines, Malaysia, China, Samoa, the Galápagos Islands, the Colombian Caribbean Sea, the Solomon Islands, and Tonga as shown in Table 2.1.

Table 2.1: The commercial value of *S. horrens* according to country

Country	Commercial value of <i>S. horrens</i>	References
Indonesia	The world's leading exporter and producer of <i>bêche-de-mer</i> provides a source of income and food.	Patantis et al. (2019)
Philippines	Overexploited fishery of <i>S. horrens</i> for economic reasons.	Bautista et al. (2017)
Malaysia	Traditional medicine: <i>gamat</i> oil and <i>gamat</i> water are used to treat wound, rejuvenate tissue and promote recovery after childbirth. Health supplements: <i>gamat</i> tonic, jelly, emulsion, essence of snakehead fish (<i>Channa striatus</i>) and <i>gamat</i> . Cosmetics: <i>gamat</i> soap, 'Gamadent' toothpaste Functional foods: dried <i>trepang</i> or <i>gamat</i> -based <i>bêche-de-mer</i> (<i>Ibu gamat</i>), frozen <i>gamat</i> , and <i>gamat</i> coffee powder.	Purcell et al. (2014); Zulfaqar et al. (2016); Kamarudin et al. (2016; 2017); Bakar et al. (2020)
China	Edible tropical sea cucumbers and traditional medicine.	Shangguan et al. (2015); Hu et al. (2013)
Samoa	Harvested for viscera (intestine, respiratory tree, and gonads). <i>S. horrens</i> viscera is consumed raw (Figure 2.1).	Eriksson et al. (2007)
Galápagos Islands	Illegal fishing and exportation of <i>S. horrens</i> for economic purposes.	Hearn and Pinillos (2006)
Solomon Islands Tonga	<i>Bêche-de-mer</i> exporter. Commercial and subsistence <i>bêche-de-mer</i> fishery. <i>S. horrens</i> is collected for its viscera (digestive tract, respiratory tree, and gonads) for local consumption and trade.	Lee et al. (2019) Charan-Dixon et al. (2019)



Figure 2.2: Bottled *S. horrens* viscera products were made of viscera from *S. horrens* (the brown mass) and the body wall of *Bohadschia vitiensis* (the white mass) (Source: Eriksson et al., 2007)

In Malaysia, *S. hermanni* is traditionally recognised for its effectiveness in treating joint pain, wound injuries, and back pain (Kamarudin et al., 2010; Manan et al., 2016; Pangestuti and Arifin, 2018). These *Stichopus* spp. or *gamat* has gained global popularity and is now available in diverse product forms. The dried and powdered form of *gamat* were used in cosmetics (lotions, ointments, soaps, creams, toothpaste, facial masks, acne cream and facial cleanser) and health supplements (pills, essences, jellies, and emulsions) (Baine and Choo, 1999; Purcell et al., 2014; Manan et al., 2016; Vaitilingon et al., 2016). Examples include Minyak Gamat Plus, a *gamat* oil manufactured by Shaari Malaysia has shown an impressive wound healing effects in Wistar albino rats (*Rattus norvegicus*), Gamadent toothpaste containing gamat extract, help treat gingivitis, while *gamat* gel (15% *S. horrens* extract) has shown similar wound-healing capabilities to Duoderm® hydrogel (Wen et al., 2018; Subramaniam et al., 2013; Taiyeb-Ali et al., 2003). Traditional *gamat* products, including *gamat* water, *gamat* essence (combined essence of snakehead fish and *gamat*), *gamat* emulsion, and *gamat* jelly are traditionally consumed to boost libido and energy, promote wound healing, reduce swelling in joints and bones, stimulate appetite, improve blood circulation, and treat haemorrhoids, asthma, diabetes, and high blood pressure. Additionally, they are believed to benefit skin health and overall well-being (Subramaniam et al., 2013; Purcell et al., 2014; Manan et al., 2016).

In addition to their value as food and medicine, sea cucumbers play a crucial ecological role in marine ecosystems. They contribute to food webs as deposit or plankton feeders, assist in nutrient cycling and benthic-pelagic coupling, and are involved in important biogeochemical processes such as bioturbation and biomineralization (Cheng et al., 2021; Mercier et al., 2025). Furthermore, sea cucumbers help increase seawater alkalinity, which mitigates ocean acidification and

supports coral health (Cheng et al., 2021; Mercier et al., 2025). They also provide habitat and food for over 200 symbiotic organisms from seven different phyla, thereby promoting biodiversity. Removing sea cucumbers could have negative consequences, including the degradation of sediment health, disruption of nutrient cycling, hindrance of organic matter transfer to higher trophic levels, and a decline in biodiversity (Mercier et al., 2025).

Sea cucumber aquaculture has significant potential in Malaysia. Zulfaqar et al. (2016) and Purcell et al. (2014) highlight that the environmental conditions (climax and water quality) along the Malaysian coastline, especially in Sabah, are suitable for aquaculture. Recent research on the medicinal properties of *gamat* continues to enhance its credibility and market potential. With *gamat* products expanding nationally and internationally, their popularity is set to rise. This creates an opportunity for further research on *Stichopus* spp. Expanding research could contribute to a prosperous future for Malaysia's *gamat* industry. By developing aquaculture for *Stichopus* spp., Malaysia could achieve profitability and meet the growing market demand for *gamat*-related products.

2.6 Life cycle of *Stichopus* sp.

Like other commercial sea cucumber, *Stichopus* spp. is dioecious and a broadcast spawner, where males and females release gametes simultaneously into the water column (Hu et al., 2013; Rahman, 2014; Purcell et al., 2023). As gonochoric broadcast spawners, sea cucumbers require close proximity for effective fertilisation of their gametes (Purcell, 2010; Purcell et al., 2011). After fertilisation, the life cycle of *Stichopus* spp. begins and their development is divided into the planktonic stage and

the benthic stage. Larvae are planktonic that feed on microalgae in the water column during the dispersive larval phase, and adults are benthic that live on the sea bottom or on hard substrates, under rocks, coral reefs, or as epizoites on plants or invertebrates.

Stichopus spp. has four larval stages: gastrula (8-25h post fertilisation), auricularia (45h-13d post fertilisation), doliolaria (18-26d post fertilisation), and pentactula (19-27d post fertilisation). After thirty days of fertilisation, the pentactula larvae changes to a juvenile (Hu et al., 2013). The embryonic and larval development of *S. horrens* is shown in Table 2.2.

Table 2.2: The embryonic and larval development of *S. horrens* at 25–27 °C with size (mean \pm SE; n=5) (Source: Hu et al., 2013)

Stage	Time	Size (μm)
Fertilisation	0	120.60 \pm 11.53
2-cell	40–50min	139.86 \pm 11.54
4-cell	70–80min	145.54 \pm 7.48
8-cell	100min	172.41 \pm 9.88
16-cell	120min	185.43 \pm 6.38
128-cell	180min	197.59 \pm 5.17
Blastula	220min	201.76 \pm 6.51
Early gastrula	8–9h	232.36 \pm 13.47
Late gastrula	25h	326.71 \pm 6.04
Early auricularia	45h	402.55 \pm 67.92
Mid auricularia	6d	502.65 \pm 71.42
Auricularia	8d	1005.44 \pm 129.45
Late auricularia	13d	1437.35 \pm 43.33
Doliolaria	18–26d	746.0 \pm 82.10
Pentactula	19–27d	398.52 \pm 7.38
Juvenile	30d	1682.32 \pm 320.58

2.7 Reproductive biology of sea cucumber

Most marine invertebrate population densities are sustained through sexually produced offspring, which are generated by broadcast spawning and the development of planktonic larvae (Thorne et al., 2012). As broadcast spawners, the fertilisation success of sea cucumbers is limited in populations that are heavily exploited. While they do not show obvious sexual dimorphism, their distinct sexes can be identified spawning by examining the released gametes or determined through biopsy to assess the gonads (Vaitilingon et al., 2016; Agudo, 2006). Fertilisation occurs externally in the water when sperm meet the eggs (Ajayi and Amedu, 2020). It has been reported that the reproductive synchrony of most sea cucumber species is controlled by the chemical communication and chemosensory systems in the sea cucumber. Males release chemicals that attract and stimulate both sexes to spawn. For example, the pheromone in male *Holothuria arguinensis* spawning water was reported to cause conspecifics and sympatric species, *Holothuria mammata* to aggregate and spawn (Marquet et al., 2018). Aggregative behaviours increase the likelihood of gamete contact and chemical exchange between individuals, which facilitates gametogenesis, spawning, and fertilisation success.

Many species of tropical aspidochirotid holothuroids are prominent asexual reproducers, propagating through transverse fission as well as broadcast spawning (Thorne et al., 2012; Hartati et al., 2019). Asexual reproduction through transverse fission is common, especially when environmental conditions are unfavourable for sexual reproduction (Uthicke, 2001; Indriana et al., 2018). *H. atra*, *S. chloronotus*, *H. pardalis*, and *S. naso* are known to be fissiparous species that reproduce asexually through transverse fission, where the resulting anterior and posterior body parts regenerate to form new individuals, emphasizing their potential for natural population

replenishment (Uthicke, 2001; Massin et al., 2007; Indriana et al., 2018; Ahmed and Ali, 2020; Hartati et al., 2019). Asexual reproduction in sea cucumbers acts as a defence mechanism in response to environmental stressors, particularly when environmental parameters fall outside the optimal range (Indriana et al., 2018). The higher fission rate in sea cucumbers is driven by factors such as increased food (or energy) availability, high mortality, a small optimum individual size in the habitat, low larval supply, and unstable habitats with significant fluctuations in salinity and temperature (Uthicke, 2001). Additionally, factors such as tidal cycles, water depth, water dynamics, and population density contribute to increased asexual reproduction in sea cucumbers (Indriana et al., 2018). High rates of transverse fission can impact the population structure by increasing densities of small individuals, elevating biomass values, and causing biased sex ratios, which may result from low recruitment or differential juvenile or larval mortality (Uthicke, 2001).

There are two types of gametogenesis in sea cucumbers: synchronous, where both males and females release mature gametes at the same time, and asynchronous, where males and females release gametes at different times (Arsad et al., 2020). For example, *H. scabra* has asynchronous gametogenesis and able to breed throughout the year (Ramofafia et al., 2003). Asynchronous gonadal development has also been reported in *S. monotuberculatus* (Cheng et al., 2021).

Echinoderms, including sea cucumbers, often have a seasonal reproductive cycle synchronised by environmental factors. Sea cucumber reproduction is notably influenced by the lunar cycle. For example, *S. horrens* in the Xisha Islands spawn during new moon days, between midnight and 2 a.m. (Hu et al., 2013). In the Philippines, *S. horrens* had its peak spawning phase 4-5 days before the new moon, occurring around midnight (22:00-02:00 h). Spawning densities reached their peak