EFFECTS OF STOCKING DENSITY AND PHOTOPERIOD ON GROWTH AND FEEDING BEHAVIOUR OF *Stichopus fusiformiossa* IN CAPTIVITY

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by

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LIST OF SYMBOLS

%	Percentage
&	And
µm	Micrometre
L	Litre
cm	Centimetre
x	Multiply
˚C	Degree Celsius
K	Kelvin
mg/L	Milligram per litre
g	Gram
$g\ d^{-1}$	Gram per day
$\%\ d^{-1}$	Percentage per day
$g\ ind^{-1}\ d^{-1}$	Gram per individual per day
m^2	Metre square
$<$	Less than
$>$	More than
±	Plus-minus
R^2	R squared
=	Equal
N	Sampling size (number of repetitions)
n	Sampling size (number of replicates)

LIST OF ABBREVIATIONS

ANOVA	Analysis of variances
CEMACS	Centre for Marine and Coastal Studies
CMC	Carboxymethyl cellulose
CV	Coefficient of variation
e.g.	Exempli gratia
FPR	Faecal production rate
GLM	Generalised linear mixed-effects
GR	Growth rate
IR	Ingestion rate
IUCN	The International Union for Conservation of Nature
LEDs	Light-emitting diodes
MCTs	Mutable collagenous tissues
PVC	Polyvinyl chloride
RWG	Relative weight gain
SEAFDEC	Southeast Asian Fisheries Development Center
SGR	Specific growth rate
sp.	Species
SR	Survival rate
USD	United States dollar
WG	Weight gain

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KESAN KEPADATAN STOK DAN FOTOKALA KEPADA PERTUMBUHAN DAN PERILAKU PEMAKANAN *Stichopus fusiformiossa* DALAM KURUNGAN

ABSTRAK

Timun laut daripada genus *Stichopus* (secara tempatannya dikenali sebagai gamat) mempunyai nilai ekonomi dan budaya yang tinggi di Malaysia, khususnya dalam perubatan tradisional dan produk kesihatan. Permintaan pasaran yang semakin tinggi telah meningkatkan tekanan terhadap penangkapan gamat, sekali gus membawa kepada penyusutan sumber dan menimbulkan kebimbangan terhadap kelestarian jangka panjang. Walaupun mempunyai nilai kepentingan, pengetahuan mengenai *Stichopus fusiformiossa* masih lagi terhad, khususnya dalam bidang akuakultur. Aspek utama seperti kepadatan stok optimum, tindak balas fotokala, prestasi pemakanan, dan ciri tingkah laku masih kurang difahami, sekali gus menghadkan kemajuan dalam amalan pengkulturan. Bagi mengatasi masalah ini, kajian ini meneliti kesan kepadatan stok dan fotokala terhadap pertumbuhan, kemandirian, serta perilaku pemakanan *S. fusiformiossa* dalam keadaan akuakultur terkawal. Dalam eksperimen kepadatan stok, lima rawatan (1, 2, 4, 6, dan 8 individu setiap tangki) diuji dengan tiga replikat selama 14 minggu. Dalam eksperimen fotokala, tiga rejim diuji: kegelapan berterusan, fotokala pendek, dan fotokala panjang, dengan lima replikat bagi setiap rawatan dan dipantau setiap jam selama tujuh hari. Untuk menambahkan kekukuhan dapatan, eksperimen fotokala ini diulangi sebanyak lima kali, menjadikan jumlah replikat sebanyak 25 bagi setiap rejim. Dalam kedua-dua eksperimen, individu dipelihara dalam sistem akuakultur tertutup dan diberi makan setiap hari dengan pelet makanan berasaskan rumpai laut. Bagi semua kepadatan stok, individu mengalami penurunan

berat badan sepanjang 14 minggu, walaupun kadar kemandirian kekal 100%. Unjuran pertumbuhan menunjukkan bahawa kepadatan dua individu mempunyai potensi terbaik untuk pemulihan, mencapai pertumbuhan positif lebih awal berbanding kepadatan lain. Kepadatan yang tinggi menyebabkan lebih banyak tindak balas tekanan (eviserasi dan penanggalan kulit) serta kehilangan berat badan yang lebih besar, tetapi menghasilkan pertumbuhan yang lebih seragam. Untuk perilaku fotokala, sekali lagi kadar kemandirian adalah 100%, dan aktiviti pemakanan didapati kebanyakannya bersifat nokturnal. Rejim cahaya mempengaruhi masa dan intensiti pemakanan: fotokala pendek meningkatkan pemakanan dan menyokong ritma malam yang stabil, fotokala panjang melambatkan penurunan nokturnal (perubahan posisi), manakala kegelapan berterusan mengganggu corak aktiviti (perubahan posisi). Tindak balas tekanan adalah minimum, dengan satu kejadian eviserasi bagi setiap rawatan dan tiada penanggalan kulit yang berlaku. Dapatan ini menunjukkan bahawa kepadatan stok dan fotokala memberi kesan ketara terhadap tingkah laku dan potensi pertumbuhan *S. fusiformiossa*, dengan kepadatan rendah serta fotokala pendek menunjukkan potensi terbaik. Walau bagaimanapun, penurunan berat badan yang berterusan dalam semua rawatan mencadangkan bahawa diet pelet berasaskan rumpai laut mungkin tidak mencukupi secara nutrisi untuk menyokong pertumbuhan positif. Kajian masa hadapan sewajarnya menumpukan kepada pengoptimuman formulasi makanan, digabungkan dengan faktor persekitaran, bagi merealisasikan sepenuhnya potensi akuakultur spesies ini.

EFFECTS OF STOCKING DENSITY AND PHOTOPERIOD ON GROWTH AND FEEDING BEHAVIOUR OF *Stichopus fusiformiossa* IN CAPTIVITY

ABSTRACT

Sea cucumbers of the genus *Stichopus* (locally known as *gamat*) hold significant economic and cultural value in Malaysia, particularly for their use in traditional medicine and health products. Rising market demand has intensified harvesting pressure, leading to resource depletion and concerns about long-term sustainability. Despite their importance, information on *Stichopus fusiformiossa* remains scarce, particularly for aquaculture development. Key aspects such as optimal stocking density, photoperiod responses, feeding performance, and behavioural traits are poorly understood, limiting progress in culture practices. To address these gaps, this study investigated the effects of stocking density and photoperiod on the growth, survival, and feeding behaviour of *S. fusiformiossa* under controlled aquaculture conditions. In the stocking density experiment, five treatments (1, 2, 4, 6, and 8 individuals per tank) were tested in triplicate over 14 weeks. In the photoperiod experiment, three regimes were applied: continuous darkness, short photoperiod, and long photoperiod, with five replicates per treatment that were observed hourly over seven days. To increase the robustness of the findings, this photoperiod experiment was repeated on five separate occasions, yielding a total of 25 replicates per treatment. In both experiments, individuals were maintained in a closed aquaculture system and fed daily with a seaweed-based pellet diet. Across all stocking densities, individuals experienced weight loss over 14 weeks, though survival remained 100%. Growth projections suggested that Density 2 had the greatest potential for recovery, achieving

positive growth earlier than other densities. High densities caused more stress responses (evisceration and shedding) and greater weight loss but resulted in more uniform growth. Under photoperiod treatments, survival was again 100%, and feeding activity was predominantly nocturnal. Light regimes influenced the timing and intensity of feeding: the short photoperiod enhanced feeding and supported stable nocturnal rhythms, the long photoperiod delayed nocturnal descent, and continuous darkness disrupted activity patterns. Stress responses were minimal, with one evisceration event per treatment and no skin shedding. These findings demonstrate that stocking density and photoperiod significantly influence the behaviour and growth potential of *S. fusiformiossa*, with low density and short photoperiod showing the greatest promise. Nonetheless, the persistent weight loss observed under all treatments suggests that the seaweed-based pellet diet may have been nutritionally inadequate to support positive growth. Future studies should therefore focus on optimising feed formulations, in combination with environmental factors, to fully realise the aquaculture potential of this species.

CHAPTER 1

INTRODUCTION

1.1 Overview

Sea cucumbers, classified under Holothuroidea within the phylum Echinodermata, are essential marine invertebrates highly valued for their economic significance and promising therapeutic applications. They are vital bioturbators in marine ecosystems, actively contributing to nutrient-cycling processes (MacTavish et al., 2012). Moreover, as benthic organisms predominantly inhabit the sea floor, they are invaluable bioindicators for monitoring metal contamination levels in marine environments (Culha et al., 2016; Marrugo-Negrete et al., 2021). Revered in Asian culinary traditions and deeply rooted in historical use in traditional medicine, sea cucumbers have garnered substantial scientific interest due to their diverse array of bioactive compounds, which offer a broad spectrum of health benefits including anticancer, antihyperlipidemic, antihyperglycemic, anti-inflammatory, anticoagulant, antioxidant, antihypertensive, antifungal, antibacterial, antimicrobial, anti-fouling, and antitumor properties (Hossain et al., 2020; Liang et al., 2022).

Among the sea cucumbers harvested in Malaysia, the genus *Stichopus*, locally known as *gamat*, holds significant importance in Malaysian aquaculture (Kamarudin et al., 2010). Malaysia hosts several species of *Stichopus*, including *Stichopus chloronotus*, *Stichopus fusiformiossa, Stichopus hermanni, Stichopus horrens, Stichopus monotuberculatus, Stichopus ocellatus*, *Stichopus rubermaculosus*, and *Stichopus vastus* (Massin et al., 2002; Woo et al., 2015). Among these, *S. chloronotus* and *S. horrens* are reported as the most encountered species. Traditionally, all *Stichopus* species have been collectively referred to as *gamat* and utilised in the production of *gamat* oil and various medicinal derivatives, valued for their purported therapeutic properties such as promoting wound healing, treating epilepsy, managing cholesterol levels, alleviating pain and burns, and enhancing overall vitality (Choo, 2004; Mazliadiyana et al., 2017; Purcell et al., 2014).

In recent decades, the increasing demand for economically valuable sea cucumber species has intensified the depletion of wild stocks due to persistent unsustainable overfishing pressures (Bordbar et al., 2011; Choo, 2004). Consequently, aquaculture has emerged as a promising restorative approach to mitigate the decline of wild sea cucumber populations through targeted restocking initiatives (Tolon et al., 2017). Notably, China has emerged as a leading global producer of sea cucumbers (Chen, 2004), effectively implementing aquaculture strategies for vital commercial species such as *Apostichopus japonicus* and *Holothuria scabra*. This shift towards aquaculture relieves pressure on wild populations and creates economic opportunities for coastal communities through sustainable culture and sea ranching practices.

Although limited sea ranching practices are in place, there is a noticeable lack of aquaculture initiatives explicitly targeting the genus *Stichopus* on a global scale. This knowledge gap within the aquaculture industry, especially regarding tropical species, is apparent. Key aspects such as feeding preferences, ethology, breeding protocols, and indoor cultivation methods for this species remain largely unexplored. This deficiency highlights the urgent necessity for comprehensive research and development efforts to address these crucial gaps and establish sustainable aquaculture practices for *Stichopus* species. Implementing optimum stocking density and understanding their natural behaviour requires attention to initiate indoor sea cucumber aquaculture practices effectively.

1.2 Problem Statements

The species *S. horrens* is an extensively exploited species of sea cucumber in Malaysia, particularly in the Langkawi and Pangkor islands. Consequently, the population in Langkawi has experienced a significant decline. Although this species is found throughout the tropical Indo-Pacific Ocean, its population status remains uncertain. It has been classified as data deficient by the IUCN Red List owing to limited knowledge of its ecology and biology (Conand et al., 2013). Increasing demand for sea cucumbers has intensified harvesting pressure, leading to the exploitation of previously unharvested species (Gamboa et al., 2004; Han et al., 2016). However, aquaculture research in Malaysia has primarily focused on *H. scabra*, leaving significant gaps in knowledge concerning other ecologically and economically important species within the *Stichopus* genus.

Accurate species identification within this genus is challenging. *S. horrens* and *S. fusiformiossa*, for example, are notoriously difficult to differentiate based solely on morphological traits and are frequently traded under the same name, *S. horrens* (Massin et al., 2002; Woo et al., 2015). As a result, much of the current aquaculture information attributed to *S. horrens* may also apply to *S. fusiformiossa*. Nevertheless, species-specific biological and ecological data for *S. fusiformiossa* remain scarce. For instance, the optimal spawning season, gonadal maturation characteristics, ideal stocking densities, interactions with environmental parameters, and hatchery production strategies are yet to be established. Information on feeding preferences, nocturnality, photosensitivity, behavioural responses, and habitat characteristics is similarly limited and primarily derived from juvenile studies (Palomar-Abesamis et al., 2017, 2018a, 2018b; Rioja et al., 2020).

As of today, there is limited knowledge of *S. horrens* for aquaculture. For instance, the optimal spawning season for *S. horrens* or characteristics of the matured gonads is still unknown. The ideal stocking density, synergical effects with environmental factors, and strategies for maximising hatchery output also remain largely unexplored. Additionally, information on feeding preferences, nocturnality and photosensitivity, behavioural responses, and habitat characteristics is limited, primarily focusing on the juvenile stage (Palomar-Abesamis et al., 2017, 2018a, 2018b; Rioja et al., 2020).

While aquaculture is essential for meeting consumer demand and conserving wild populations, challenges such as unsynchronised growth and unclear growth rate–density relationships persist. Furthermore, studies addressing the effects of environmental factors such as temperature and photoperiod on locomotion and feeding behaviour remain scarce, thereby constraining the optimisation of mariculture technologies. The present study, therefore, investigates the effects of stocking density and photoperiod on the feeding performance, growth rate, and survival of *S. fusiformiossa*. Addressing these gaps is critical for developing efficient, environmentally sustainable aquaculture strategies tailored to *Stichopus* species, thereby improving growth, reproduction, and overall productivity in culture systems.

1.3 Research Objectives

The objectives of this study are to:

1) Determine the effects and optimal stocking density on the growth and survival of sea cucumber, *S. fusiformiossa*, in captivity.
2) Investigate the effects of different photoperiod exposure on the growth and feeding behaviour of sea cucumber, *S. fusiformiossa*, in captivity.

CHAPTER 2

LITERATURE REVIEW

2.1 Fisheries Overview

The global trade and economics of sea cucumber fisheries play a pivotal role in shaping both local economies and international markets. Sea cucumbers, members of the class Holothuroidea, are highly prized for their culinary and medicinal value, particularly in Asian countries such as China, Hong Kong, and various nations in Southeast Asia (Purcell et al., 2012). Hong Kong dominates the Asian import market, acquiring approximately 56 million kilograms of sea cucumbers from 2012 to 2019, representing 63% of the region's overall imports. Mainland China follows with 21%, while Malaysia, Singapore, and the Taiwan Province of China account for 7%, 4%, and 4% respectively. Other Asian importers, like the Macao Special Administrative Region of China, Vietnam, and Myanmar, make up the remaining 1% (Louw & Bűrgener, 2020). The demand for sea cucumbers in these regions has driven a significant global trade market, encompassing both dried and fresh sea cucumbers (Conand, 2008). Various factors, including species diversity, size, quality, and cultural preferences, influence the trade in sea cucumbers. Different species of sea cucumbers command different prices in the market, with some species being more highly valued than others due to perceived taste or medicinal properties (Conand, 2008). Additionally, the size and quality of sea cucumbers can affect their market value, with larger and higher-quality specimens often fetching higher prices (Purcell et al., 2013, 2018).

In Southeast Asia, sea cucumber fisheries are integral to the economies of coastal communities, providing employment opportunities and income for local fishers and traders. Traditional fishing methods, such as hand gathering and diving, are commonly employed in these regions, mainly where sea cucumbers are abundant

(Purcell et al., 2013). These traditional methods often involve small-scale operations run by local communities, contributing to the socio-economic fabric of coastal areas. However, industrial fishing operations also play a significant role in sea cucumber fisheries alongside conventional fishing methods, especially in regions with high demand and commercial interest. Industrial methods such as bottom trawling, dredging, and the use of suction pumps enable the harvesting of sea cucumbers in large quantities, catering to the demands of global markets (Conand, 2008). While these industrial methods may increase efficiency and productivity, they also pose risks to marine ecosystems through habitat destruction and overexploitation.

The economic importance of sea cucumber fisheries underscores the need for sustainable management practices to ensure the long-term viability of these resources. Promoting sustainable fisheries includes implementing regulations and quotas to limit fishing pressure and establishing protected areas to safeguard critical habitats (Purcell et al., 2013). Additionally, initiatives to promote alternative livelihoods and sustainable aquaculture practices offer opportunities to reduce pressure on wild populations while meeting market demand (Conand, 2008). In conclusion, the global trade and economics of sea cucumber fisheries are complex and multifaceted, with implications for both local economies and marine ecosystems. Sustainable management practices are essential to balance economic interests with conservation goals and ensure the continued availability of sea cucumbers for future generations.

2.2 Sea Cucumber Aquaculture

Sea cucumber aquaculture has undergone rapid global expansion, driven largely by increasing demand from Asian markets such as China, Korea, and Taiwan, where these species are highly valued for both culinary and medicinal purposes (Bordbar et al., 2011). More than 70 species are harvested worldwide, with *Apostichopus japonicus* (Japanese sea cucumber) and *Holothuria scabra* (sandfish) representing the most heavily exploited taxa (Purcell et al., 2013). Overfishing of wild stocks, fuelled by high market prices, has led to substantial resource depletion, thereby necessitating the adoption of sustainable aquaculture practices (Purcell et al., 2013). Among the strategies proposed, integrated multi-trophic aquaculture (IMTA) systems, where deposit-feeding sea cucumbers are co-cultured with other species, have been advocated to optimise resource utilisation and mitigate aquaculture waste (Zamora et al., 2016).

Despite these developments, large-scale commercial applications of IMTA incorporating sea cucumbers remain concentrated in China, with other regions still limited to pilot-scale operations (Han et al., 2016; Zamora et al., 2016). Moreover, the industry faces considerable risks from disease outbreaks and environmental stressors such as climate change. *A. japonicus* has been notably susceptible to mass mortality events, highlighting the vulnerability of cultured stocks (Yu et al., 2023; Zhao et al., 2022). These challenges underscore critical research gaps in pathogen management, environmental sustainability, and the understanding of species-specific ecological roles within aquaculture systems.

Historically, Japan and China have been at the forefront of sea cucumber aquaculture, particularly through extensive research on *A. japonicus* in temperate waters and *H. scabra* in tropical regions. Breeding and rearing trials date back to the 1930s (Yellow Sea Fisheries Research Institute, 1991), and this sustained effort has enabled the successful commercialisation of both species (Choo, 2008). *H. scabra* remains the predominant tropical species cultured, with limited success reported for other taxa (Purcell et al., 2012). This narrow focus has been attributed to the species' favourable traits, including its tolerance to salinity fluctuations (Tuwo et al., 2021), as well as strong market demand. Such adaptability has facilitated its introduction into temperate countries to meet sustainability goals and satisfy economic demand (Purcell et al., 2012).

Within Southeast Asia, the aquaculture of sea cucumbers has become increasingly prominent, particularly in Indonesia, the Philippines, and Vietnam. Among these, Indonesia has emerged as a major producer, relying on both wild populations and hatchery-based culture to meet the growing demand from the Chinese market (Han et al., 2016; Rahman et al., 2020). Advances in hatchery and grow-out techniques have contributed to greater production stability, with *H. scabra* remaining the dominant cultured species owing to its high economic value. Integrated farming systems, which are increasingly adopted in Indonesia, have further enhanced the ecological and economic sustainability of aquaculture practices (Han et al., 2016; MacTavish et al., 2012).

Building on these developments, Indonesia now represents one of the most biodiverse regions for sea cucumbers, with approximately 350 species of sea cucumbers have been documented, with at least 26 recognised as commercially important, primarily from the families *Holothuriidae* and *Stichopodidae* (Pangestuti & Arifin, 2018). Hatchery and farming initiatives have expanded in response to unsustainable harvesting, with species such as *Stichopus chloronotus* exhibiting year-round reproductive activity in areas like Karimunjawa National Park (Mustagfirin et al., 2021). Despite these advances, key challenges remain, including limited understanding of pathogen risks such as antimicrobial resistance in *Vibrio parahaemolyticus* (Jiang et al., 2014) and insufficient nutritional studies to support the development of functional food applications (Fawzya et al., 2015).

Similar progress has been reported in Thailand, where aquaculture production of sea cucumbers has risen markedly, estimated at around 130,000 tonnes annually (Robinson et al., 2019). Research efforts have focused on refining farming techniques, including polyculture systems that integrate sea cucumbers with scallops and jellyfish to enhance both productivity and ecological sustainability. Organic waste from aquaculture has also been explored as a supplementary food source, improving growth and survival, particularly during high-stress summer months when mass mortality events are common (Yu et al., 2023). Nevertheless, uncertainties persist regarding the long-term ecological effects of intensified culture practices and the genetic diversity of cultured stocks, both of which are crucial to disease prevention and production stability (Wang et al., 2021; Yu et al., 2023).

In the Philippines, which ranks second globally after Indonesia in sea cucumber production with an estimated 20,000 tonnes annually (Ereguero et al., 2022), similar trends have been observed. Approximately 100 species are commercially exploited, of which 25 are most actively traded (Arriesgado et al., 2022). Overfishing and weak regulatory frameworks have led to sharp population declines, emphasising the urgency of aquaculture as an alternative. Hatchery success has been achieved for *Holothuria scabra*, notably at facilities such as Mindanao State University, although survival of early juveniles in field grow-out systems remains low (Magcanta et al., 2021). Current research emphasises the importance of developing management approaches that integrate ecological science with local community participation (Fabinyi et al., 2022; Purcell et al., 2016), yet nutritional and bioactive studies remain scarce, limiting opportunities for value-added products and enhanced consumer markets (Pangestuti & Arifin, 2018).

In Malaysia, the current status of sea cucumber research underscores the urgent need for effective management and sustainable practices in response to significant population declines. Approximately 40 species have been identified, with *Holothuria leucospilota*, locally known as *Patola*, being one of the most abundant (Harith et al., 2018). Overfishing driven by high demand in Asian markets has caused severe depletion of commercially important species, with coastal areas reporting substantial declines in biomass and endemic species considered particularly vulnerable (Anderson et al., 2011; Baker-Médard & Ohl, 2019). Although some advances have been made, such as studies on microorganisms associated with local species that may support aquaculture health management (Solehin et al., 2024), several research gaps remain. These include inadequate data on ecological impacts of harvesting, insufficient genetic research to support selective breeding, and limited assessments of climate change effects on population dynamics (Léopold et al., 2013; Rawson & Hoagland, 2019). Furthermore, socio-economic evaluations of local communities engaged in sea cucumber fisheries are scarce, which constrains the development of inclusive management strategies (Purcell et al., 2016).

The commercial value of sea cucumbers in Southeast Asia varies considerably, with high-value species like *H. scabra* and *S. horrens* commanding significant market interest (Bordbar et al., 2011; Purcell, 2014; Purcell et al., 2014). While farming in the region is primarily centred on H. scabra, the diversity of economically valuable species is notable (Choo, 2008). In Malaysia, *S. horrens* is one of the most harvested species, although historically it has held lower commercial value in Chinese markets (Chen, 2004). Over the past decade, however, its market price has increased substantially, particularly in Hong Kong and Guangzhou, reflecting shifts in demand and supply chains (Purcell et al., 2018). Market analyses in Southeast Asia categorise sea cucumber species into three value tiers: low, moderate, and high, encompassing around 15 species (Tuwo & Tresnati, 2015). Due to rising demand, evolving market preferences, and overharvesting, some species previously considered low-tier have attained prices comparable to high-value species, and additional species have entered the market by 2022 (SEAFDEC, 2022). Among these, *S. horrens* now ranks among the most valuable (Table 2.1).

Table 2.1 Comparison of sea cucumber market value categories (Tuwo & Tresnati, 2015) and local price ranges (SEAFDEC, 2022)

Scientific name	**Common name**	**Market value tier (2015)**	**Local price (USD/kg, 2022)**
Actinopya echinites	Deepwater redfish	Low	12–54
Bohadschia vitiensis	Brown sandfish	Low	NA
Holothuria atra	Lollyfish	Low	NA
Holothuria edulis	Pinkfish	Low	NA
Holothuria fuscopunctata	Elephant trunkfish	Low	NA
Thelenota anax	Amberfish	Low	4–51
Actinopyga mauritiana	Surf redfish	Moderate	NA
Actinopyga miliaris	Hairy blackfish	Moderate	NA
Bohadschia argus	Leopardfish	Moderate	9–27
Holothuria nobilis/ Holothuria whitmaei	Black teatfish	Moderate	17–105
Holothuria scabra	Sandfish	High	30–105
Holothuria fuscogilva	White teatfish	High	17–88
Stichopus chloronotus	Greenfish	High	NA
Stichopus horrens	Dragonfish (*gamat*)	High	24–58
Thelenota ananas	Prickly redfish	High	12–51
Actinopyga lecanora	Stonefish	NA	7–66
Stichopus hermanni	Curryfish	NA	58

Note: NA = not available, data not reported for this species in the respective source.

Notwithstanding these advances, the region continues to face persistent challenges in governance, resource management, and ecological sustainability. Continued fishing pressure on wild stocks further underscores the urgent need for targeted research on species diversification, refined hatchery protocols, effective disease prevention strategies, and robust environmental impact assessments (Anderson et al., 2011; Han et al., 2016).

In Malaysia, the development of sea cucumber aquaculture has progressed more slowly compared to neighbouring Southeast Asian nations, with production historically reliant on wild capture fisheries (Choo, 2008; Chen, 2004). Initial farming efforts primarily targeted *H. scabra*, supported by relatively well-established hatchery protocols and consistent high market demand (Purcell et al., 2012). Interest in *S. horrens* emerged in 1994 (Eeckhaut, 2009) due to its abundance in local waters and expanding export potential. However, large-scale culture remains limited, owing to challenges in broodstock conditioning, larval rearing, and grow-out performance (Cheong et al., 2024). Addressing these bottlenecks requires not only advances in hatchery and grow-out technologies but also the development of optimised feeding strategies. In this context, dietary research, particularly the use of seaweed-based formulations has gained increasing attention as a means to enhance growth, survival, and sustainability in sea cucumber aquaculture.

2.3 Seaweed-Based Diets in Sea Cucumber Aquaculture

Seaweeds have been recognised not only as valuable nutritional supplements but also as contributors to enhanced growth rates and survival in juvenile sea cucumbers. Species such as *Sargassum thunbergii* and *Ulva lactuca* have been tested for their effects on juveniles of *Apostichopus japonicus*. It was demonstrated by Anisuzzaman et al. (2017) that diets incorporating different algae resulted in variable growth and health outcomes, with *Sargassum* producing particularly favourable effects on growth performance and immune response. Similarly, Setiawati et al. (2021) confirmed that the combination of fresh benthos with *Sargassum* and *Ulva* improved both growth and reproductive performance in *Stichopus* spp.

The inclusion of *Spirulina* as a dietary supplement for sea cucumbers has been increasingly investigated due to its high protein content, antioxidant properties, and potential health benefits. Studies have demonstrated that *Spirulina* can effectively support the growth of various aquaculture organisms, including juvenile sea cucumbers, and its nutritional value positions it as a promising candidate for enhancing aquaculture efficiency (Windari et al., 2019). More recently, it was shown that diatoms cultured in association with macroalgae, including *Spirulina*, can serve as a nutritious feed for *Stichopus japonicus* juveniles (Jiang et al., 2020). When combined with traditional diets, *Spirulina* is expected to improve overall health and growth efficiency by providing essential nutrients beyond those supplied by conventional feeds (Botutihe et al., 2024).

In tropical species, the sustainability of aquaculture is closely linked to dietary quality and composition. Xu et al. (2022) examined *Stichopus monotuberculatus* in a sea ranching context and reported that enriched diets containing *Sargassum* enhanced growth performance. Their findings highlighted the potential of sea ranching to alleviate pressure on wild populations while maintaining optimal growth through dietary improvements. Similarly, Zacarías-Soto et al. (2018) demonstrated that dietary formulations incorporating diverse seaweeds significantly enhanced growth and improved the biochemical composition of *Isostichopus badionotus*.

The incorporation of seaweed in diets has also been associated with improvements in environmental sustainability. Sediment enrichment with seaweeds such as *Sargassum* was shown by Hou et al. (2017) to facilitate food uptake while simultaneously improving the sedimentary environment of cultured sea cucumbers. Beyond nutritional benefits, the use of seaweeds as feed ingredients reduces reliance on formulated diets and contributes to the development of more ecologically responsible aquaculture systems. This sustainability perspective is particularly relevant given the current overexploitation of many tropical species, which necessitates the adoption of responsible aquaculture practices (Arriesgado et al., 2024).

In summary, the inclusion of seaweeds, such as *Sargassum* and *Ulva,* in sea cucumber diets has been shown to enhance growth performance, promote ecological sustainability, and strengthen aquaculture practices. Continued exploration of seaweed-based formulations is therefore expected to contribute substantially to the recovery and long-term stability of sea cucumber populations. To fully realise these benefits, however, dietary improvements must be complemented by advancements in culture systems. Recent progress in the design and application of recirculating and semi-recirculating aquaculture systems has provided promising strategies for optimising production efficiency, enhancing environmental control, and supporting the sustainable expansion of sea cucumber aquaculture.

2.4 Recirculating and Semi-Recirculating Indoor Aquaculture Systems

Aquaculture systems are generally classified into open systems, such as cages and ponds, which depend on natural water flow from the surrounding environment, and closed systems, which operate without direct water exchange. In recirculating aquaculture systems (RAS), more than 90–99% of the water is continuously treated and reused through mechanical and biological filtration, oxygenation, and disinfection, allowing high-density production with minimal environmental discharge and precise control of physico-chemical parameters (Goddek et al., 2015; Martins et al., 2010). These advantages contribute to improved growth performance and biosecurity; however, the high capital investment, technical expertise, and operational costs required can limit their application in small-scale or research settings. Production costs in RAS have been estimated at USD 1.2–1.6 per kg of fish, compared to USD 0.6–0.7 per kg in conventional systems (Asiri & Chu, 2020).

Semi-recirculating aquaculture systems can minimise water use and waste production, offering ecological advantages comparable to full RAS but without the high financial burden (Ngọc et al., 2016). While RAS can improve growth rates and feed conversion efficiencies, high operational costs often constrain small-scale operations, making semi-recirculating systems an attractive middle-ground that combines efficiency with cost-effectiveness (Senff et al., 2020; Ngọc et al., 2016). Compared to full RAS, semi-recirculating systems require greater water exchange to dilute waste metabolites, yet still reduce nutrient loading relative to conventional flow-through systems (Ramli et al., 2020). Their simplicity, lower infrastructure demands, and adaptability make them well-suited for experimental aquaculture and small-scale hatcheries. In some cases, they can also be integrated with aquaponics or polyculture approaches to enhance resource-use efficiency (Nederlof et al., 2022).

2.5 The Effects of Stocking Density in Sea Cucumber Aquaculture

Stocking density in aquaculture refers to the number of organisms, such as fish or invertebrates, maintained within a given volume of water or culture area. It is recognised as a critical parameter influencing growth, health, and overall productivity in cultured species. The optimal stocking density varies according to species and culture system and must be established to maximise production while minimising stress and disease incidence (Arifin et al., 2017).

The effects of stocking density are multifaceted. Higher densities can intensify competition for food and space, leading to reduced growth rates, poorer feed conversion, and elevated stress levels (Millán-Cubillo et al., 2016). Water quality may deteriorate as a result of waste accumulation, thereby increasing disease susceptibility. Conversely, excessively low densities can result in the underutilisation of available resources, limiting productivity and economic returns (Millot et al., 2024). Achieving a balanced stocking density is therefore essential for optimising growth performance and maintaining animal welfare (Jia et al., 2022).

In sea cucumber aquaculture, stocking density plays a significant role in the growth and health of the organisms (Tolon et al., 2017). Research has shown high stocking densities can result in adverse outcomes such as stunted growth, increased mortality, and deformities (Qin et al., 2009). For instance, studies on *A. japonicus*, a widely cultured species, have demonstrated that overcrowding can cause slow growth rates and high body size variability, negatively impacting marketability and profitability (Qin et al., 2009). Moreover, optimal stocking densities are essential to prevent issues such as 'rotten stomach' syndrome, which has been linked to poor environmental conditions in high-density settings (Qin et al., 2009). Similar concerns apply to *H. scabra* and *Stichopus* spp., where density optimisation is crucial not only for biomass yield but also for maintaining system stability, particularly in integrated multitrophic aquaculture (IMTA) systems, where beneficial interactions with species such as oysters and sea squirts can enhance growth (Kim et al., 2019; Zamora et al., 2016). A summary of stocking density effects across species is presented in Table 2.2.

Despite the potential benefits of IMTA, high-density cultures present management challenges, including increased organic waste and declining water quality (Granada et al., 2016). Adjustments to operational parameters, such as the carbon–nitrogen ratio, have been shown to improve nutrient assimilation and mitigate waste effects in semi-intensive systems (Gao et al., 2016). Such integrative approaches can sustain sea cucumber viability across different densities, though site-specific environmental conditions remain decisive in determining the optimal range (Zamora et al., 2016).

In Malaysia, the rapid expansion of sea cucumber aquaculture has heightened the risk of overcrowding, leading to slower growth and greater disease incidence. However, research on stocking density in indoor recirculating aquaculture systems is still limited. Continuous environmental monitoring and density adjustment remain essential for maximising growth and ensuring welfare. The influence of other environmental factors, such as photoperiod, must also be considered in combination with density to fully optimise culture conditions, as discussed in the following section.

Table 2.2 Summary of reported effects of stocking density on growth and survival of various sea cucumber species

Species	Stocking Density (ind m^{-2})	Key Findings	Reference
Apostichopus japonicus	5, 10, 15, 25, 35	Growth rate significantly decreased with increasing density	(Qin et al., 2009)
Holothuria scabra	10, 20, 30, 40, and 3, 6, 9, 12	Lower densities yielded higher growth; survival unaffected	(Lavitra et al., 2010)
Apostichopus japonicus	1, 2, 4, 6, 8	Growth rate, feed conversion efficiency, and energy allocation declined with density	(Pei et al., 2012)
Holothuria tubulosa	6, 15, 30	Growth and weight gain highest at lowest density; declined sharply at highest density	(Tolon et al., 2017)
Holothuria scabra	150, 250, 500, 700, 1000, 2000	Survival, weight, and growth decreased significantly at higher densities	(Altamirano & Noran-Baylon, 2020)

2.6 Phototaxis and Nocturnality

Phototaxis, the movement of organisms in response to light stimuli, plays a critical role in the behaviour and ecological adaptation of tropical sea cucumbers, including *S. fusiformiossa*. Many species within the class Holothuroidea exhibit negative phototaxis, characterised by an aversion to light and a preference for darker environments. This behaviour functions as a key survival mechanism, enabling sea cucumbers to avoid predation and reduce exposure to potentially harmful environmental conditions (Li et al., 2019). In the case of *S. fusiformiossa*, a pronounced tendency to inhabit shaded areas such as natural crevices or, in captivity, beneath artificial substrates like PVC has been observed. Such behaviour has also been documented in closely related species, including *S. monotuberculatus*, *A. japonicus*, and *H. scabra*, all of which display strong avoidance of illuminated areas (Chen et al., 2022; Li et al., 2019).

Closely linked to negative phototaxis is nocturnality, another adaptive trait widely observed among sea cucumbers. Numerous species, including *H. scabra*, and *A. japonicus* have been observed to increase their locomotor and feeding activities during nighttime hours, when light intensity is minimal and predator visibility is reduced (Dong et al., 2010; Sun et al., 2018). Peak feeding behaviour in *A. japonicus*, for example, has been observed during the early hours of the night, supporting the hypothesis that nocturnality serves to enhance foraging success while minimising predation risk (Sun et al., 2018). Environmental variables such as water temperature and current velocity have been shown to influence the efficiency of nocturnal feeding and movement, thereby enhancing the energy balance and growth potential of sea cucumbers (Chen et al., 2022; Sun et al., 2018). For instance, *S. monotuberculatus* has been reported to exhibit elevated locomotor and feeding activities during nighttime under relatively high temperatures, with a preference for downstream areas characterised by low water velocity (Chen et al., 2022).

Nocturnality is a prominent characteristic of many tropical sea cucumber species, including *H. scabra* and *A. japonicus*, which exhibit heightened activity during the night for feeding and locomotion (Sun et al., 2018). The nocturnal feeding patterns are likely an evolutionary adaptation that allows them to exploit food resources while minimising predation risk. For example, peak feeding activity in *A. japonicus* has been documented during the early hours of the night, coinciding with reduced visibility for potential predators (Sun et al., 2018). Environmental factors such as water temperature and flow velocity may also enhance locomotion and feeding efficiency during these hours (Chen et al., 2022; Sun et al., 2018). Understanding the phototaxis and nocturnal behaviours of tropical sea cucumbers provides valuable insights for optimising environmental conditions in aquaculture systems, particularly lighting and feeding schedules, to promote growth, health, and overall production efficiency.

2.7 Identification of *Stichopus fusiformiossa*

Accurate identification within the genus *Stichopus* has been hindered by considerable morphological similarities among species, resulting in frequent taxonomic challenges. Species such as *Stichopus fusiformiossa*, *Stichopus horrens*, *Stichopus monotuberculatus*, and *Stichopus naso* often share overlapping external characteristics, leading to potential misidentification when relying solely on morphology, as illustrated in Figure 2.1 (Fan et al., 2011; Juinio-Meñez et al., 2023; Woo et al., 2015). The challenge in identifying such closely related species increases significantly due to the variability of morphological traits, which is influenced by environmental factors, geographical distribution, and the ontogenetic development of these organisms. This issue is further complicated by the presence of highly variable colouration and body morphology within a single species. For instance, both *S. monotuberculatus* and *S. horrens* are known to exhibit differing colour patterns in nature depending on their locality (Byrne et al., 2010; Gray et al., 2023; Lizano et al., 2024). As a result, these species have often been traded under the same name, typically *S. horrens*, despite representing distinct taxa.

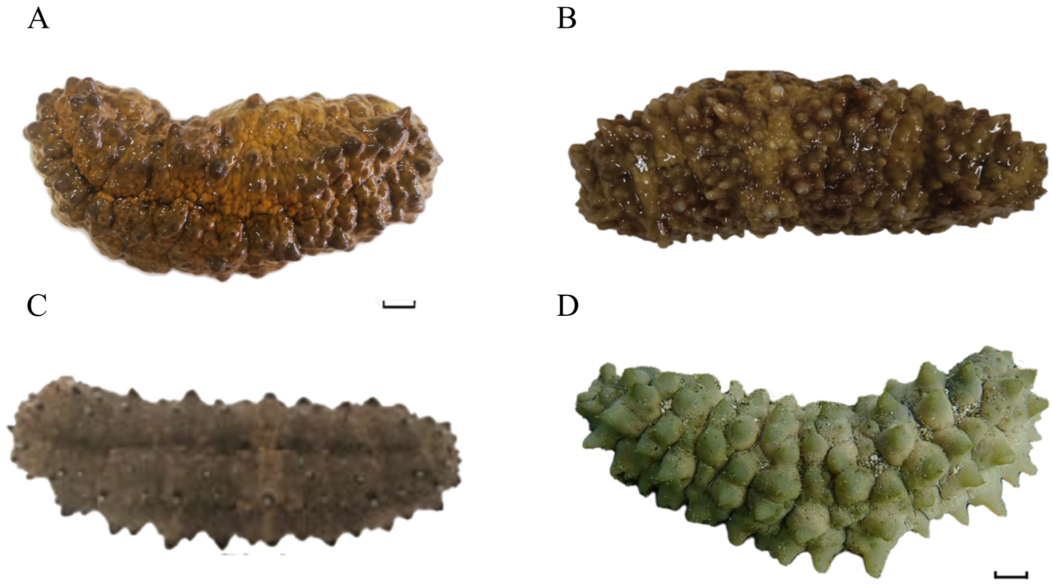

Figure 2.1 External morphology of four *Stichopus* species commonly misidentified as *Stichopus horrens*. A: *Stichopus fusiformiossa*. B: *Stichopus horrens* (reproduced from Torreno et al., 2023). C: *Stichopus monotuberculatus* (adapted from Zhong et al., 2023). D: *Stichopus naso* (adapted from Nishihama et al., 2020). Scale bar = 1 cm, where applicable

One method for identifying sea cucumber species involves examining the ossicles, which are small calcareous structures found in the body of holothurians. These ossicles serve both a structural role and a pivotal function in taxonomic classification, as they are unique to each species and exhibit distinct morphological characteristics. The ossicle morphology can provide critical insights into the specific identity of the sea cucumber being examined, making it a cornerstone technique in taxonomy (Akashah et al., 2021).

Ossicles are typically extracted from multiple anatomical regions, including the dorsal body wall, papillae tips, tentacles, and tube feet (Teoh & Woo 2021). Small tissue samples were treated with commercial bleach to dissolve organic material, thereby releasing the calcareous ossicles (Stöhr et al., 2008). The remaining ossicles were then thoroughly rinsed multiple times with distilled water to eliminate any residual chemicals. Cleaned ossicles were then mounted onto glass slides for microscopic observation (Teoh & Woo 2021).

Microscopic examination was conducted using a light microscope, and, where higher resolution was required, a scanning electron microscope (SEM) was employed. This enabled the detailed observation of morphological traits such as size, shape, and surface ornamentation of the ossicles, which serve as key features used in species identification. Particular attention was paid to the diversity of ossicle types, such as tables, rods, and rosettes (Figure 2.2), as well as the structural details of their surfaces, which may vary significantly among species (Fan et al., 2011; Woo et al., 2015).

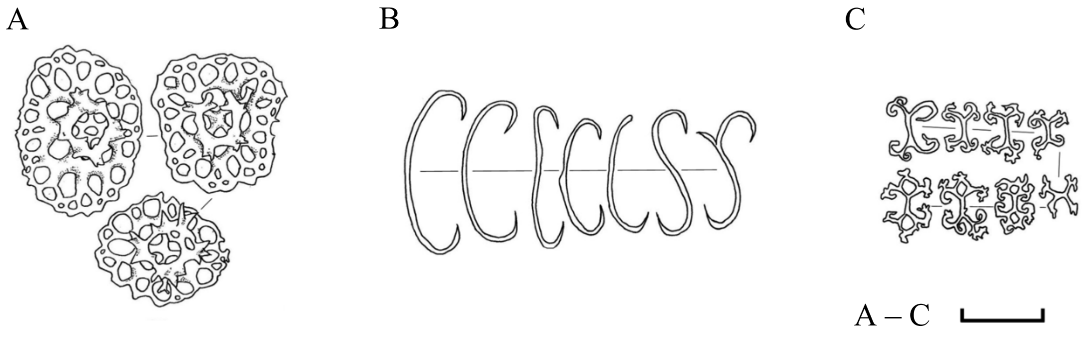

Figure 2.2 Examples of three types of ossicles extracted from the papillae of *Stichopus fusiformiossa*. A: table with large base plates. B: C-shaped rods. C: rosettes. These represent a subset of the ossicle diversity observed in this species. Scale bar = 0.1 mm (adapted from Woo et al., 2015)

By adhering to established methods of ossicle examination, a more reliable identification of sea cucumber species can be achieved, including *S. fusiformiossa*, despite the morphological similarities it shares with closely related species, particularly *S. horrens*. In the present study, the specimens were found to lack the tack-like table ossicles in the papillae, a definitive taxonomic feature of *S. horrens*, with distinct fusiform ossicles observed in the tentacles (Figure 2.3). Based on these morphological characteristics and in accordance with the species description provided by Woo et al. (2015), the sea cucumber specimens examined in this study were identified as *S. fusiformiossa* (Figure 2.4).

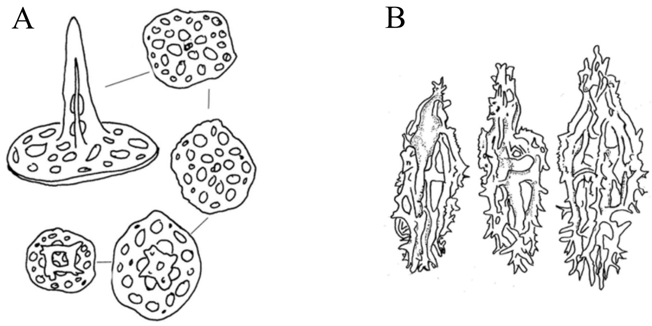

Figure 2.3 Comparison of ossicles relevant to identification. A: tack-like table ossicles, absent from the papillae of the examined specimen but characteristic of *Stichopus horrens*. B: fusiform ossicles, present in the tentacles of the examined specimen (adapted from Woo et al., 2015)