EFFECT OF DIATOM DIETS ON THE GROWTH OF JUVENILE SEA CUCUMBER *Holothuria scabra*

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BORNEO MARINE RESEARCH INSTITUTE
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2022

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2022

UNIVERSITI MALAYSIA SABAH

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DECLARATION

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DEGREE	:	**MASTER OF SCIENCE**
FIELD	:	**AQUACULTURE**
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ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor Associate Professor Dr. Sitti Raehanah Muhamad Shaleh for her continuous support, expert guidance, understanding and encouragement throughout my study. Without her guidance and persistent help, this dissertation would not have been possible.

Special thanks were also expressed to the Ministry of Higher Education for the financial support throughout this study. This study was funded under the Niche Research Grant Scheme (NRGS0002) and Fundamental Research Grant FRG0469-2017.

I also wish to express my sincere thanks to all the faculty members of Borneo Marine Research Institute for providing me with all the necessary facilities, help and encouragement. Thank you also to my comrades who have been and are still on the same journey for their knowledge sharing and emotional support. Finally, I would like to thank my parents and family members for their continuous moral support and unconditional encouragement all this while.

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ABSTRACT

Holothuria scabra, also known as sandfish, is a highly valued sea cucumber that is widely farmed in the Indo-Pacific region. However, farmed sandfish seeds were taken from the wild habitat, resulting in population depletion. As a result, production in hatcheries is being established to meet the needs of aquaculture. While diatoms are widely cultured and used in bivalves and sea cucumber hatcheries. Adequate diet is critical for hatchery production because it has a direct impact on growth and survival. In this study, to achieve the aim of understanding the appropriate diet for sandfish juveniles, *Chaetoceros gracilis* were cultured using agricultural fertilizer (NPK) and harvested using chitosan through flocculation. Diatom *Navicula* sp. the microalgal flocs produced were fed to juvenile sandfish to determine the growth performance. In objective 1, the growth of *C. gracilis* was tested in different types of media consisting of 5 concentrations of fertilizer (NPK 10:8:6) and Walne's Media as the control. Final cell densities (cells/mL), specific growth rate (cell/d), doubling time, and division rate (div/day) was determined. The findings revealed that liquid fertilizer at 0.5 mL/L has the highest final cell counts and relatively high specific growth rate (7.428±0.05 10^6 cells/mL and 1.201±0.05 cell/day respectively). For objective 2, determination of flocculation efficiency (%) of *C. gracilis* harvested using chitosan was done under different pH (7, 8, and 9), salinity (20, 25, 30, and 35 ppt), chitosan concentration (0 – 75 ppm) and cell density (0.5, 2, 4, and 6 cells/mL). Based on the findings, *C. gracilis* harvested in 20 ppt, pH 9, cell density of 4 x 10^6 cells/mL with chitosan concentration of 75 ppm showed the highest percentage of flocculation efficiency (89%). In objective 3, the growth performance of juvenile sandfish was determined by first testing the ingestion and digestion of 2 diatom diets (*Navicula* sp. and *C. gracilis* flocs) and a 2-week feeding trial of 6 experimental diets consist of combination of *C. gracilis* flocs and sea mud with different inclusion levels of microalgal flocs (N, CF0, CF10, CF20, CF30, CF100). *Navicula* sp. (diet N) was a control diet. Ingestion rates were determined by observing the faecal excretion time and cell wall digestion of microalgae was determined by viewing newly excreted faeces under a fluorescent microscope. While, for the feeding trials, approximately 1 cm juvenile sandfish were placed in small plastic containers with a stocking density of 0.01 juveniles/cm^2. Initial and final weight was measured to determine the weight gain (%), specific growth rate (%/day), and condition factor (%). The findings showed that both *Navicula* sp. and *C. gracilis* were ingested by the juvenile sandfish. However, *C. gracilis* showed high cell wall digestion while *Navicula* sp. was observed to be indigestible. The feeding trial of experimental diets showed that juvenile sandfish fed with CF30 had higher weight gain and specific growth rate (59.20±27.25% & 3.25±1.22%/d respectively) followed by CF100 (35.17±10.32% & 1.83±0.83%/d) and CF20 (30.02±5.59% & 1.87±0.31%/d). However, there were no significant differences between diet CF20, CF30, and CF100 in weight gain (.244), specific growth rate (.451), or survival (.339). This study demonstrates the efficiency of chitosan as flocculant for flocculation harvesting of *C. gracilis* and the viability of using *C. gracilis* flocs as feed to juvenile sandfish to promote growth and survival. It is also advised to combine sea mud and *C. gracilis* flocs in a 70:30 ratio

for improved growth performance and less diatom biomass required for diet preparation.
Keywords: aquaculture, sea cucumber, growth, diet, marine diatom

ABSTRAK

KESAN DIET MICROALGA TERHADAP PERTUMBUHAN BENIH TIMUN LAUT Holothuria scabra

Holothuria scabra atau umum dikenali sebagai bat atau balat adalah salah satu spesies timun laut yang mempunyai nilai yang tinggi dan diternak secara meluas di rantau Indo-Pasifik. Walau bagaimapun, benih balat yang diternak adalah diambil dari habitat semulajadi dan ini mengakibatkan pengurangan populasi balat. Oleh itu, penghasilan benih di hatceri telah dilaksanakan untuk menampung keperluan akuakultur. Diatom pula dikultur dalam kuantiti yang banyak untuk diijadikan makanan di hatceri penetasan kerang dan timun laut. Untuk penghasilan di hatceri, diet yang mencukupi adalah sangat penting kerana ia memberi kesan secara langsung terhadap pertumbuhan dan kemandirian balat. Dalam kajian ini, demi memahami pemakanan yang sesuai untuk benih balat, Chaetoceros gracilis telah dikultur menggunakan baja pertanian NPK dan dituai menggunakan kitosan melalui teknik bio-flokulasi. Diatom dan flok mikroalga yang dihasilkan diberi makan kepada balat untuk menentukan prestasi pertumbuhan balat. Dalam objektif 1, pertumbuhan C. gracilis diuji dalam lima kepekatan baja pertanian (0.5, 1, 2, 3, dan4 mL/L) dan media Walne sebagai kawalan. Kepadatan sel akhir (sel/mL), kadar pertumbuhan spesifik (sel/hari), masa penggandaan, dan kadar pembahagian (div/day) ditentukan dalam eksperimen ini. Hasil kajian menunjukkan bahawa baja cecair NPK 0.5 mL/L mempunyai bilangan sel akhir yang paling tinggi dan SGR yang agak tinggi (7.428 ± 0.05 10^6 sel/mL dan 1.201 ± 0.05 sel/hari) berbanding dengan rawatan lain. Dalam objektif 2, eksperimen dilakukan untuk menentukan kecekapan flokulasi (FE) C. gracilis menggunakan kitosan dalam keadaan tertentu iaitu salinity (20, 25, 30, dan 35 ppt), pH (7, 8, dan 9), kepekatan kitosan (0 – 75 ppm) dan kepadatan sel (0.5, 2, 4, and 6 cells/mL). Berdasarkan penemuan tersebut, C. gracilis dalam salinti 20 ppt, pH 9, kepadatan sel 4 x 10^6 sel/mL dengan kepekatan kitosan 75 ppm menunjukkan peratusan kecekapan flokulasi tertinggi (89%). Dalam Objektif 3, prestasi pertumbuhan balat juvenil ditentukan dengan terlebih dahulu menguji pengambilan dan pencernaan 2 diet diatom (Navicula sp. dan flok C. gracilis) dan percubaan pemberian makanan selama 2 minggu untuk 6 diet eksperimen terdiri daripada gabungan flok C. gracilis dan lumpur laut dengan peratusan flok yang berbeza (N, CF0, CF10, CF20, CF30, CF100). Navicula sp. (diet N) ialah diet kawalan. Kadar pengambilan ditentukan dengan memerhatikan masa perkumuhan najis manakala pencernaan dinding sel mikroalga ditentukan melalui pemerhatian pada najis baharu yang terkumuh menggunakan mikroskop fluoresen. Sementara itu, untuk ujian pemberian makanan, balat juvenil yang berukuran kira-kira 1 cm ditempatkan di tangki eksperimen berukuran 0.03 m^2 dengan kepadatan stok 0.01 juvenil/cm^2. Berat awal dan akhir diukur untuk menentukan peningkatan berat badan (%), kadar pertumbuhan spesifik (%/d), dan condition factor (%). Hasil kajian menunjukkan bahawa kedua-dua diet iaitu Navicula sp. dan flok C. gracilis dimakan oleh balat juvenil, namun C. gracilis menunjukkan pencernaan dinding sel yang tinggi sementara Navicula sp. didapati tidak dapat dicerna. Ujian diet eksperimen menunjukkan bahawa balat juvenil yang diberi makan CF30 mempunyai peningkatan berat badan dan kadar pertumbuhan spesifik yang lebih tinggi

(masing-masing 59.20 ± 27.25% dan 3.25 ± 1.22%/d) diikuti oleh CF100 (35.17 ± 10.32% dan 1.83 ± 0.83%/d) dan CF20 (30.02 ± 5.59 % dan 1.87 ± 0.31%/d). Namun, tidak ada perbezaan yang signifikan antara semua diet eksperimen dari segi peningkatan berat badan (.244), kadar pertumbuhan spesifik (.451) dan kemandirian (.339) balat juvenil. Kajian ini menunjukkan kebolehlaksanaan penggunaan flok C. gracilis sebagai makanan pada peringkat juvenil untuk pertumbuhan dan kemandirian. Selain itu disarankan juga untuk mencampurkan lumpur laut bersama flok C. gracilis dengan nisbah 70:30 kerana prestasi pertumbuhan yang lebih baik dan penggunaan biomass mikroalga yang sedikit dalam penyediaan diet.
Kata kunci: akuakultur, timun laut, pertumbuhan, diet, diatom marin.

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LIST OF SYMBOLS

%	-	Percentage
kg	-	Kilogram
USD	-	United States dollar
g	-	Gram
cm	-	Centimetre
sp.	-	Species
L	-	Litre
°C	-	Degree celsius
g/L	-	Gram per litre
µm	-	Micrometre
mL	-	Millilitre
cells/mL	-	Cells per millilitre
rpm	-	Revolutions per minute
N	-	Normality
mg	-	milligram
µl	-	microlitre
Mg/mL	-	Milligram per millilitre
µg	-	microgram
mm	-	Millimetre
g/m	-	Gram per cubic metre
OD	-	Optical density
ppm	-	Part per million
ppt	-	Part per thousand

LIST OF ABBREVIATIONS

ADC	-	Apparent Digestibility Coefficients
ADR	-	Apparent Digestibility Ratio
a.m	-	*Ante meridiem* (morning)
ANOVA	-	Analysis of Variance
AVS	-	Acid-volatile Sulfur
EDTA	-	ethylenediaminetetraacetic acid
CF	-	Condition Factor
FAO	-	Food and Agriculture Organization of United Nations
FCD	-	Final cell density
FCR	-	Faeces Production Ratio
FE	-	Flocculation efficiency
HDPE	-	High-density polyethene
IR	-	Ingestion Rate
IUCN	-	International Union for Conservation of Nature and Natural Resources
n/a	-	Not available
pH	-	Potential hydrogen
p.m	-	*Post meridiem* (afternoon)
SE	-	Standard error
SEAFDEC	-	Southeast Asian Fisheries Development Centre
SGR	-	Specific Growth Rate
SPSS	-	Statistical Package for Social Sciences
UMS	-	Universiti Malaysia Sabah
UV	-	Ultraviolet
WG	-	Weight gain
w/v	-	Weight over volume

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CHAPTER 1

GENERAL INTRODUCTION

1.1 World Aquaculture Industry

The world's capture fisheries are about 75% overfished. In order to secure future fish production, global fisheries have evolved from capture fisheries to aquaculture in recent years (Huntington & Hasan, 2009). According to reports from the Food and Agriculture Organization of United Nations (FAO) (2020), most of the world's fisheries production was based on fish caught in the wild, but due to population growth and urbanization, aquaculture programs began to gain popularity. Each year, aquaculture production volume increases at an average rate of 5.3%, doubling from 25.7% in 2000 to 46% in 2018. Compared to other parts of the world, Asia (excluding China) is responsible for most of the growth in fish availability through aquaculture, accounting for 42% of global fish production.

Malaysian aquaculture is promoted as a vital industry for the country's economy. With a total production of 521,000 tonnes, the country is one of the top 15 aquaculture producers in the world. However, as one of the largest fish eaters in the world, annual fish landings are insufficient to meet the needs of the population (Yusoff, 2015; Fathi *et al.*, 2018). In Malaysia, capture in fisheries accounts for 82.70% of the total fisheries production while aquaculture accounts for only 17.01%. Intensive fishing activities have led to the depletion of wild fish resources. Therefore, the enforcement of aquaculture in Malaysia is necessary to solve the current problems and challenges of the aquaculture industry, including the ever-increasing demand for fish and the decline of wild-caught fish due to overfishing. In recent years, freshwater and brackish water aquaculture have dominated fisheries production. In 2019, aquaculture production of freshwater and

brackish water fish totaled 223,671.74 metric tonnes (Department of Fisheries, 2019). The major species cultured in freshwater were black and red tilapia (*Oreochromis* spp.), freshwater catfish (*Clarias* sp.), riverine catfish (*Pangasius* sp.), and freshwater giant prawn (*Macrobrachium rosenbergii*). The main species farmed in brackish water are cockles (*Anadara granosa*), marine prawns (*Penaeus monodon* and *P. vannamei*), marine finfish, mussels (*Perna ciridis*) and other crustaceans. Most of these species are the staple food in Malaysia, besides other marine species are farmed for medicine, food, and export, such as sea cucumber.

1.2 Sea cucumber Aquaculture

Sea cucumbers are a high-value product in global seafood markets. They have been harvested without restriction and overexploited, resulting in their populations becoming almost non-existent in some places and listed as an endangered species by the International Union for Conservation of Nature Resources (IUCN) (Hamel *et al.*, 2013). There are not many sea cucumber hatcheries, most of the *bêche-de-mer* produced are wild-caught by local farmers or traded with neighbouring countries to be processed into *bêche-de-mer* when they reach maturity (Baine & Forbes, 1998).

The earliest sea cucumber aquacultures have been operated in Japan since 1977, in India in 1988, in China in 1990 and in the Maldives in 1997 for breeding and stock enhancement. These countries are some that have successfully adopted hatchery techniques that enable them to produce massive annual seed production; China (>6 billion), Maldives (>5 million) and Japan (>3 million). The Maldives is the only country that produces *Holothuria scabra*, while the other country produces *Aposthichopus japonicus* (Purcell *et al.*, 2012a). On the other hand, in Southeast Asia, they are a few countries with reported seed productions hatcheries which are Vietnam (*H. scabra*), Philippines (*H. scabra*, and *Stichopus horrens*), and Palau (*Actinopyga mauritiana* and *Actinopyga miliaris*). However, there are not many hatcheries still around and the remaining hatcheries that produce *H. scabra* are only in Vietnam and the Philippines (Purcell *et al.*, 2012a, Tuwo & Tresnati, 2015).

In Malaysia, there are more than 80 species of sea cucumber (Kamarul Rahim et al., 2010), of which two are considered highly valued species that are processed for medicinal purposes and exported to other countries, namely gamat (*Stichopus horrens*) and balat putih or sandfish (*Holothuria scabra*). Gamat is cultivated in West Malaysia, while balat putih is found mainly in Sabah. Balat putih, also known as sandfish, is one of the most highly valued tropical species in Asian markets. The price of dried sandfish varies depending on the size and can reach up to 251 - 1898 USD per kilogram (Purcell, et al., 2018). In Sabah, sandfish is found in Kunak, Semporna, Kudat and Tuaran (Baine & Choo, 1999, Kamarul Rahim *et al.*, 2009, Arsad *et al.*, 2020). However, there are no restocking programs in Sabah and enforcement of fisheries policy and regulatory measures may not be sufficient to address the problem of declining sandfish populations.

In captive rearing of sandfish, the phase between larvae and juveniles is crucial (Purcell, 2012a), as the animals require constant and nutritious food for growth and survival. Many studies have been conducted on the diet of juvenile sea cucumbers, with most studies focusing on the species *Aposthicopus japonicus*, which is found in Japan, Korea, and China (Huiling *et al.*, 2004; Seo *et al.*, 2011a; Liao *et al.*, 2015). The experimental size of the animals studied is 1 - 5 g juveniles. A few studies have also examined formulated diets and co-cultures on tropical sea cucumbers (Giraspy & Ivy, 2008; Purcell *et al.*, 2006; Pattinasarany, et. al., 2014), but no studies have examined the feasibility of feeding microalgae flocs to small sandfish juveniles weighing less than 1 g and the effects on their growth and survival.

1.3 Significant of Study

Sandfish farming indirectly plays an important role in the Malaysian economy and marine ecology. Extensive research on juvenile rearing strategies is required to ensure the success of farming this species. In 2004, FAO highlighted the importance of sea cucumber stock enhancement and compiled several status reports and reviews on sea cucumber fisheries, trade, farming, and aquaculture (Lovetelli *et al.*, 2004). Much research has been conducted to better understand

and improve hatching procedures, diseases, and especially the nutrition of sea cucumbers.

In the wild, the ocean ecosystem is rich in inorganic and organic matter, including microalgae. It is only natural that the diet of sandfish in the wild also consists of microalgae. According to Rashidi *et al*., (2018), the gut content of a sea cucumber consisted of three parts: diatoms/microalgae, algae and benthos. Microalgae are nutrient-rich microorganisms that provide a vital diet during the larval stage and early juvenile stage of sandfish (Knauer, 2011; Duy *et al.*, 2017). A study by Duy *et al.* (2017) using live microalgae and concentrated microalgae in feeding juvenile sandfish found that some types of microalgae are highly digested by juvenile sandfish, some are moderately or indigestible. For example, microalgae such as *Chaetoceros muelleri* were highly digested by juvenile sandifish while microalgae such as *Isochrysis* 1800® and *Pavlova* 1800® were moderately digested and *Tetraselmis* 3600® was indigestible. It is essential to understand which types of microalgae feed are accepted by sandfish in order to maintain their growth and survival during their early stages of life.

Sandfish are bottom feeders and use their tentacles to grab food and pull it into their mouths when food is nearby (Hamel & Mercier, 1998). Some microalgae, e.g., *Chaetoceros* sp. are small microalgae that float in the water column. Therefore, it is important to use a harvesting method that allows the microalgae to settle to the bottom. Flocculation is one of the few harvesting techniques for microalgae that use flocculants to aggregate suspended microalgae so that large aggregates either float or are suspended to the bottom, making them easier to collect (Renault *et al.*, 2009). The type of flocculant is also an important factor when feeding juvenile sandfish as the flocculant must be non-toxic so that it does not harm the juvenile fish, e.g., chitosan. Chitosan is a non-toxic material made from crustacean exoskeleton (Kurita, 2006). Another example for an environmentally friendly flocculant is through biofloc technology (BFT). The BFT is a flocculation technology that is used to improve water quality, waste treatment, and prevention of disease in intensive aquaculture systems, and the product is called bioflocs. The biofloc is made up of algae, bacteria, and protozoa that are bound together in a matrix with particle organic matter (El-Sayed, 2020). The