LARVAL DEVELOPMENT OF SANDFISH (*Holothuria scabra*) UNDER DIFFERENT FEEDING REGIME

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ABSTRACT

Sandfish (*Holothuria scabra)* is one of the most widely cultured sea cucumber species in the Indo Pacific regions. Unfortunately, most of the sandfish farmer relies almost entirely on the wild seeds. Therefore, hatchery seed production is now underway to meet the needs of aquaculture. Understanding the feeding regime is very crucial because it influenced the growth performance and affects the yield in the hatchery. In this study, three experiments were conducted with three objectives, (1) to determine the developmental period of different stages of sandfish larvae, (2) to determine the effects of different diet and stocking density and (3) to determine the effects of different feeding regimes on the growth performance of sandfish larvae. Fertilized eggs used in this study were obtained via artificial and natural spawning. The larval developmental period was determined by observing the larvae daily. Six growth stages were recorded; gastrula (24 hours after fertilization), early auricularia (day 2), mid auricularia (day 4), late auricularia (day 6), doliolaria (day 21) and pentactula (day 26). To achieve the second objective, *Nannochloropsis* sp. and *Chaetoceros calcitrans* were tested to three different stocking densities (1, 1.5 and 2 larvae ml^{-1}) on the 2 days after hatching (dAH) larvae in a factorial experiment. The experiment was carried out using 4.5 L plastic containers randomly arranged in a 2 tonnes HDPE tank, used as water bath with the temperature controlled at 29 to 30°C. The findings revealed the larval growth was significantly affected ($p<0.05$) by the diet, but not significantly affected ($p>0.05$) by the stocking density. A single species diet of *Nannochloropsis* sp. at 1.5 larvae ml^{-1} demonstrated the highest growth (109.69±8.21%). This study also demonstrated a highly significant interaction ($p<0.05$) between diet and stocking density on the larval growth. However, the survival was not significantly affected ($p>0.05$) by the diet and stocking density. There was a negative interaction ($p>0.05$) between diet and stocking density on the survival of the larvae. Although the highest survival was in the larvae fed on *Nannochloropsis* sp. at 2 larvae ml^{-1} (1.2±1.05%), but the metamorphosis was observed on day 12. On the other hand, *C. calcitrans* at 2 larvae ml^{-1} demonstrate the fastest (day 6) and highest metamorphic percentage compared to the other diets. Based on the results, five feeding regimes and two feed concentrations were tested in a factorial experiment. There was a significant interaction ($p<0.05$) between feeding regime and feed concentration on the growth of the larvae. N2C feeding regime at 4×10^4 cells ml^{-1} demonstrates the highest growth (38.09± 6.6%). The growth was significantly affected ($p<0.05$) by the feeding regime, but not significantly affected ($p>0.05$) by the feed concentration. Similarly, the survival was also significantly affected ($p<0.05$) by the feeding regime but not significantly affected ($p>0.05$) by the feed concentration. The highest survival was recorded in the larvae fed with a single feeding regime of *Nannochloropsis* sp. at 2×10^4 cells ml^{-1} (1.37±0.13%). However, there was a negative interaction ($p>0.05$) between these two factors on the survival of the larvae. Establishment of the larval rearing techniques of sandfish, particularly in the optimization of feeding regime is important as it influences the growth performance and yield of hatchery produced sandfish.

ABSTRAK

PERKEMBANGAN LARVA TIMUN LAUT PUTIH (Holothuria scabra) DALAM REGIM MAKANAN YANG BERBEZA

Balat putih (Holothuria scabra) merupakan spesis timun laut yang paling banyak dipelihara di rantau Indo Pasifik. Malangnya, kebanyakan penternak timun laut bergantung sepenuhnya kepada benih liar. Oleh itu, penghasilan benih timun laut di hatceri dilakukan untuk memenuhi keperluan akuakultur. Pemahaman tentang regim makanan amat penting kerana ia mempengaruhi prestasi pertumbuhan larva dan memberi kesan kepada penghasilan di sesebuah hatceri. Dalam kajian ini, tiga eksperimen dijalankan dengan tiga objektif untuk (1) Menentukan tempoh perkembangan setiap peringkat larva timun laut, 2) Menentukan kesan jenis makanan dan kepadatan pelepasan dan 3) Menentukan kesan regim makanan terhadap prestasi pertumbuhan larva. Telur yang tersenyawa yang digunakan dalam kajian ini diperolehi melalui pembiakan artifisial dan semulajadi. Tempoh perkembangan larva ditentukan melalui pemerhatian yang dijalankan setiap hari. Enam peringkat tumbesaran telah direkodkan; gastrula (1 hari selepas persenyawaan), awal auricularia (hari ke 2), pertengahan auricularia (hari ke 4), lewat auricularia (hari ke 6), doliolaria (hari ke 21) dan pentactula (hari ke 26). Untuk mencapai objektif kedua, Nannochloropsis sp. dan Chaetoceros calcitrans telah diuji pada tiga kepadatan pelepasan larva yang berbeza (1, 1.5 dan 2 larva ml^{-1}) dalam eksperimen faktorial ke atas larva berumur 2 hari selepas menetas. Eksperimen dijalankan menggunakan bekas plastik 4.5 L yang disusun secara rawak dalam tangki HDPE 2 tan yang digunakan sebagai rendaman air dan suhu dikawal pada 29-30°C. Hasil dapatan menunjukkan tumbesaran larva dipengaruhi secara signifikan ($p<0.05$) oleh diet, tetapi tidak dipengaruhi secara signifikan ($p>0.05$) oleh kepadatan pelepasan. Tumbesaran tertinggi (109.69±8.21%) direkodkan oleh diet Nannochloropsis sp. pada kepadatan pelepasan 1.5 larva ml^{-1}. Kajian menunjukkan terdapat interaksi yang sangat signifikan ($p<0.05$) antara diet dan kepadatan pelepasan kepada tumbesaran larva. Namun, peratus hidup tidak dipengaruhi secara signifikan ($p>0.05$) oleh diet dan kepadatan pelepasan. Terdapat interaksi negatif ($p>0.05$) antara diet dan kepadatan pelepasan kepada peratus hidup larva. Walaupun larva yang diberi makan Nannochloropsis sp. pada kepadatan pelepasan 2 larva ml^{-1} mempunyai peratus hidup tertinggi (1.2±1.05%), tetapi metamorfosis hanya berlaku pada hari ke 12. Manakala, C. calcitrans pada kepadatan pelepasan 2 larva ml^{-1} menunjukkan metamorfosis yang lebih cepat (hari ke 6) dan peratusan metamorfosis yang lebih tinggi berbanding jenis diet lain. Berdasarkan keputusan ini, lima regim makanan dan dua kepekatan makanan diuji dalam eksperimen faktorial. Terdapat interaksi yang signifikan ($p<0.05$) antara regim makanan dan kepekatan makanan kepada tumbesaran larva. Tumbesaran tertinggi direkodkan (38.09±6.6%) pada regim makanan N2C (kepekatan makanan: 4×10^4 sel ml^{-1}). Tumbesaran larva dipengaruhi secara signifikan ($p<0.05$) oleh regim makanan, tetapi tidak dipengaruhi secara signifikan ($p>0.05$) oleh kepekatan makanan. Peratus hidup juga dipengaruhi secara signifikan ($p<0.05$) oleh regim makanan tetapi tidak dipengaruhi secara signifikan ($p>0.05$) oleh kepekatan makanan. Peratus hidup tertinggi direkodkan *pada regim makanan Nannochloropsis sp. pada kepekatan makanan 2×10^4 sel ml^{-1} (1.37±0.13%). Namun, terdapat interaksi negatif ($p>0.05$) antara dua faktor tersebut kepada peratus hidup larva. Pengukuhan teknik penternakan larva timun laut, khususnya dalam mengoptimumkan regim makanan adalah penting memandangkan ia mempengaruhi prestasi pertumbuhan dan penghasilan timun laut di hatceri.*

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LIST OF ABBREVIATIONS

AIZ	Aquaculture Industrial Zone
ANOVA	Analysis of Variance
BSA	Bovine serum albumin
dAH	Days after hatched
EDTA	ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
fe	Fertilization envelope
GSS	Gonad stimulating substance
HDPE	High density polyethylene
IUCN	International Union for Conservation of Nature and Natural Resources
n/a	Not available
ODEC	Outdoor Development Centre
OMI	Oocyte maturation inductor
pH	Potential hydrogen
SE	Standard error
SPSS	Statistical Package for Social Sciences
TCA	Trichloroacetic acid
UMS	Universiti Malaysia Sabah
UV	Ultraviolet
w/v	Weight over volume

LIST OF SYMBOLS

%	Percentage
Kg	Kilogram
US$	United States dollar
G	Gram
N	North
E	East
cm	Centimetre
sp	Species
L	Litre
°C	Degree celsius
g/L	Gram per litre
μm	Micrometre
ml	Millilitre
ml^{-1}	Per millilitre
rpm	Revolutions per minute
w/v	Weight over volume
N	Normality
mg	milligram
μl	microlitre
mg ml^{-1}	Milligram per millilitre
μg	microgram
nm	Nanometre
M	Molarity
mm	Millimetre
g m^{-3}	Gram per cubic metre
ppm	Part per million
ppt	Part per thousand

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CHAPTER 1

INTRODUCTION

1.1 Global Fisheries and Aquaculture

Fishery sector plays an important role as a major supplier of animal protein worldwide. Fishery is a unit that responsible in the management of raising and harvesting fish which includes purpose of the activities, people involved, species or type of fish, method of fishing, class of boats and area of water or seabed (Marine Stewardship Council, 2017). It consists of two main components which are marine capture fisheries and aquaculture (FAO, 2009). Seafood is a main protein source for the global population and the demand shows an increasing trend with the growing population worldwide. Seafood is mostly preferred source of protein as it is cheaper and healthier compared to other animal protein source. It is also widely available and accessible as 70% of the earth is covered by water. The source of seafood majorly depending on the marine capture fisheries which covered almost 88% of the total production (FAO, 2009).

Malaysia has been listed as one of the highest consumers of fish in the world. According to Yusoff (2015), fish consumption pattern among Malaysian indicated, about 20% of their food expenditure was spent on fish. The fish consumption index in this country is expected to increase up to 15% from 53.1 kg in 2011 to 61.1 kg in 2020. The pressure on the increasing demand for fish locally and globally resulted in severe depletion of the wild caught landings. Hence, it is very important to expand the aquaculture activities to fulfil the increasing demand worldwide.

In Malaysia, aquaculture is an important engine of growth and the mainstay of the nation's economy. Aquaculture is a sector that involved in the raising of aquatic organisms within a particular partitioned aquatic area under controlled environmental conditions for the purpose of commercial scale production (Jadhav, 2008). Aquaculture sector recorded annual production of 525,000 tons, which need to be raised up to 790,000 to meet the projected demand by the year 2020. This has led to the Malaysian Government undertaking various efforts to improve aquaculture in the country. The efforts including introduction of the Aquaculture Industrial Zone (AIZ) Program which emphasizing on the development of potential zone for downstream activities such as fish seed production, fish processing plant and feed mills (Yusoff, 2015). The aim of this program is to identify constraints and issues faced by aquaculture sector, such as the increasing demand for fish and declining of the wild caught fish due to overexploitation. Shark, salmon, tuna, spiny lobster, horseshoe crab, bivalve mollusc and sea cucumber are among the most overexploited species worldwide.

Overexploitation of sea cucumbers especially Holothurians is not a current issue, as this species have been the most favourite luxury food item in the Asian dried seafood market for centuries especially in China, Taiwan and Korea (Rahman, 2014). Fisheries of this species started in 1700s in the central of Indo Pacific which further expanded worldwide (Eriksson *et al.*, 2012). According to Ram and Southgate (2014), at least 58 species are harvested and traded around the world with the main target species of *Holothuria scabra*, *H. lessoni*, *H. fuscogilva*, *H. nobilis*, and *H. whitmaei*. Sea cucumbers have high protein (43%) and low fat content (2%) (Morgan, 2000b). In addition, sea cucumbers have high therapeutic and medicinal properties such as it acts as a tonic and traditional remedy for asthma, hypertension, constipation, arthritis, wounds (Chen, 2003; Wen *et al.*, 2010; Rahman, 2014).

Sandfish was listed among the most highly valued tropical sea cucumber species in Asian markets. The price of the dried sandfish can reach up to US$115 to 640 per kilogram (Purcell *et al.*, 2012). It contains many bioactive substances which exhibit the antibacterial, antifungal and anticancer properties (Caulier *et al.*, 2013). In Malaysia, production of dried sandfish (*beche de mer*) are actively carried out in Sabah. This species can be found mainly in Kunak, Kudat and Semporna (Baine and Choo, 1999). However, this sector depends solely on the wild caught sandfish seeds.

According to Annual Fisheries Statistics, 174 tonnes of sea cucumbers were landed in Sabah in 2014 (Department of Fisheries, 2014)

Sandfish has also been listed as endangered species by the International Union for Conservation of Nature and Natural Resources (IUCN) (Hamel *et al.*, 2013). The population of sandfish declined at least 50% over the past 30 to 50 years (Hamel *et al.*, 2013). Increasing demand and continuous exploitation of sandfish for export leads to the extinction and depletion of broodstocks (Pakoa *et al.*, 2012). Enforcement of fisheries governance and regulatory measure alone may not sufficient to overcome the issue on the declining population of the highly valued sandfish (Purcell *et al.*, 2012). Therefore, expanded aquaculture activities of sandfish are necessary for the sustainability of this species.

1.2 Sandfish Aquaculture

First artificial production of sandfish was carried out by James (1988) in India in 1988. The artificial production of sandfish was carried out using the thermal stimulation method, where the temperature of the sea water was raised by 3 to 5°C above ambient temperature. Since then, sandfish aquaculture started to develop and expand all over the world. Other countries such as Maldives, Vietnam, Solomon Island, Indonesia and Australia started to produce juvenile sandfish based on the same technology developed by James (1988). Artificial production of other species of sea cucumbers such as *Apostichopus japonicus* also carried out in China and Japan. This species has been successfully produced through artificial method more than 60 years ago in Japan (Inaba, 1937).

Sandfish is the most commonly cultured species and bred extensively because it has high commercial value, widely distributed and currently the only tropical species that can be mass produced in the hatchery system (Mazlan and Hashim, 2015). Sandfish has the benefits especially in aquaculture due to its ability to be induced throughout the year compared to the other species which can only be induced once or twice a year as the spawning season was not influenced by the lunar periodicity (Che and Gomez, 1985). Hatchery production of sandfish is seen as a way to restore the depleted wild stock through the stock enhancement program called sea ranching

(Battaglene, 1999). Thus, enabling continuous seed production of sandfish throughout the year by artificial spawning.

However, there are some challenges in the hatchery seed production of sandfish especially from larval stage to the juvenile culture and broodstocks management. Larval culture is the crucial aspect and the most critical stages in aquaculture. The main challenge in the larval culture is the low survival of planktonic larvae up to the juvenile stage (Dhert *et al.*, 2001). The survival rate of the early larval stage (auricularia) up to juvenile stage commonly around 1% (Purcell *et al.*, 2012). According to Battaglene (1999) and Ramofafia *et al.* (2003), the highest mortality commonly recorded at the first feeding, hatching, metamorphosis and settlement stage. There are many factors that influenced the success of the sandfish seed production such as the quality of broodstocks, fertilization rate, water quality, feeding management, space availability and hatchery practice such as larval rearing techniques.

1.3 Significant of Study

Intensive studies on the larval rearing techniques is necessary to ensure the success in the hatchery production of this species. Guisado *et al.* (2012) stated that the studies on the reproduction, development and feeding behaviour in captivity was important for a better understanding on the life history of a particular species. Thus, the potential of the species in aquaculture and accurate strategy in terms of stock management can be determined. There are several exogenous factors that affect the larval culture of sandfish in hatchery such as diet types, stocking density, temperature and salinity (Crisp, 1974).

An appropriate feeding strategy to each stage of growth is vital to enhance the growth performance, stabilize the water quality, minimize feed wastage and to improve the efficiency of hatchery production (Dwyer *et al.*, 2002; Tucker *et al.*, 2006). Stocking density is also important factors in the aquaculture production because it directly gives impact on the growth, survival, feeding, quality and yields of cultured organisms (Rainuzzo *et al.*, 1997; Liu *et al.*, 2002; Rowlanda *et al.*, 2006). Sui (2004) reported highest mortality in early rearing stage, basically due to the overcrowding of the larvae. All these factors must be optimized in the larval rearing stage to obtain maximum growth and survival from larval stage up to the juvenile stage.

According to Battaglene (1999), the depleted wild stocks can be restored by releasing the juvenile sea cucumbers produced in hatcheries through a process called restoration, reseeding or restocking. Sandfish is the best candidate for the sea ranching because it is a well established culture species and threaten by the extinction. The recovery of the wild stocks also contributes to the sustainability of the marine ecosystem, especially in the seagrass bed ecosystem. Overexploitation of sandfish also gives negative impacts on the productivity of seagrass systems (Wolkenhauer *et al.*, 2010). Sandfish plays an important ecological role by ingesting the sediments (bioturbation) and releasing ammonia which would be usable by the seagrass bed and other nearby marine organisms. Restoration of the depleted wild stocks also improves the socio economic level of the coastal community.

1.4 Objectives of Study

The aim of this study was to establish the larval rearing techniques of sandfish in hatchery system. Larval rearing is the crucial stage and the key factor for the success in the seed production of sandfish. There are many factors determining the success of the larval rearing of sandfish such as temperature, feeding regime, stocking density and salinity. This study emphasized on the optimization of the feeding regime and stocking density in the larval rearing of sandfish. Thus, to achieve the aim of this study, the specific objectives were outlined as below:

a) To determine the developmental period of different stages of sandfish larvae.

b) To determine the effects of different diet and stocking density on the growth performance of sandfish larvae.

c) To determine the effects of different feeding regimes on the growth performance of sandfish larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 *Holothuria scabra*

Holothuria scabra, commonly known as sandfish. It is one of the invertebrate community members that occurs in all of the major oceans and seas of the world (Purcell *et al.*, 2016). Sandfish was mainly found in the Indo Pacific countries, from east Africa to the eastern Pacific. There were normally found between the latitudes of 30°N and 30°S (Agudo, 2006). Due to wide geographical distribution, *H. scabra* have many specific common names throughout its geographic distribution (Table 2.1).

Table 2.1: Common names of *Holothuria scabra*

Location	Common names	References
Malaysia	Trepang, Putih, Tepuak	Sakthivel and Swamy, 1994; Biusing, 1997; Forbes *et al.*, 1999
Vietnam	Ñoät traéng	SPC, 2014
Philippines	Cortido, Curtido, Kagisan, Rebothal, Patos, Dalamogon	Baird, 1974; Kanapathipillai and Sachithananthan, 1974; Trinidad Roa, 1987; SPC, 2014
Fiji	Dairo	Adams, 1992; Adams *et al.*, 1994; SPC, 2014
New Caledonia	Le gris, Holothurie de sable	SPC, 2014
Japan	Namako	Sakthivel and Swamy, 1994
Madagascar	Zanga fotsy, Bemavo, Tricot, Zanga mena	Sakthivel and Swamy, 1994