UNIVERSITI PUTRA MALAYSIA

GROWTH PERFORMANCE OF SLIPPER-CUPPED OYSTER (*Crassostrea iredalei* FAUSTINO, 1932) LARVAE AND SPAT FED WITH DIFFERENT MICROALGAE

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By

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Interest on oyster culture is increasing worldwide. In Malaysia, slipper-cupped oyster, *Crassostrea iredalei* is preferred as potential commercial species due to its sweet flavor and creamy colored meat. However, at the moment, oyster culture is still dependent on natural seed collection. Seed can only be collected for a short period during certain seasons with unpredictable recruitment. Therefore, the production of seedling from hatchery is essential for the sustainable production of oyster. Further research should be carried out to determine the growth and survival of oyster larvae produced in hatchery. This study specifically addressed the feeding aspect of oyster in hatchery. Three species of microalgae used in this study were *Isochrysis* sp. *Pavlova* sp. and *Chaetoceros* sp.. They were fed to larvae (D-stage and umbo stage) and spat of *C. iredalei*.

Effects of feeding three microalgae species on the growth of D-stage and umbo stage larvae *C. iredalei* were investigated. One-day-old larvae (after fertilization) were reared for six days, while seven-day-old larvae (after fertilization) were reared for fourteen days. Diet using *Isochrysis* sp. supported the highest ($p<0.05$) growth while *Chaetoceros* sp. showed the lowest growth for D-stage larvae. Larvae fed with diet *Isochrysis* sp. showed the highest final, increment of shell length and growth (85.45 ± 7.72 µm, 22.07 ± 7.72 µm, 0.05 ± 0.02 µm/day, respectively).

Meanwhile, diet combination of flagellate and diatom supported better growth for umbo stage larvae. Combination diet *Pavlova* sp. and *Chaetoceros* sp. gave the highest ($p<0.05$) growth, compared to combination of *Isochrysis* sp. and *Pavlova* sp. (lowest growth). Combination of *Pavlova* sp. and *Chaetoceros* sp. showed the highest final, increment of shell length and growth (265.89 ± 22.50 µm, 184.13 ± 22.50 µm, 0.08 ± 0.01 µm/day, respectively). Survival of D-stage and umbo stage larvae in every diet showed no significant differences. These results suggested D-stage larvae need *Isochrysis* sp. for better growth. While at umbo stage, diets containing combination of flagellate and diatom are better for the growth of this species.

The effect of feeding three microalgae species on the early development of *C. iredalei* spat was conducted. Early spats were reared for three weeks in container. Spat in all treatments survived well since there were no mortality. There was no significant difference ($p>0.05$) between diet *Isochrysis* sp./*Chaetoceros* sp. (IC) and *Pavlova* sp./*Chaetoceros* sp. (PC). Both binary diets show high significant difference ($p<0.05$) toward diet *Chaetoceros* sp. (C). Diet IC showed highest final, increment of shell length and growth (6.240 ± 0.186 mm, 2.740 ± 0.193 mm, 0.0276 ± 0.002 mm/day, respectively). Diet C supported the lowest of final, increment shell length and growth (5.413 ± 0.486 mm, 1.947 ± 0.365 mm, 0.0211 ± 0.003 mm/day, respectively). As for fatty acid level, *Isochrysis* sp. showed high content of docosahexanoic acid (DHA), *Pavlova* sp. rich in saturated fatty acid (SFA) while *Chaetoceros* sp. rich in polyunsaturated fatty acid (PUFA), eicosapentanoic acid (EPA) and arachidonic acid (AA). The lack of DHA in diet C resulted in low growth of spat. Thus, this result suggested the combination of diatom and flagellate produced better growth for early stage of *C. iredalei* spat.

As a conclusion, at the early stage of larvae development, *Isochrysis* sp. is essential as compared to diatom since it can be easily digested. However, as larvae growth, they need combination of flagellate and diatom.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KADAR TUMBESARAN LARVA DAN SPAT TIRAM (*Crassostrea iredalei* FAUSTINO, 1932) DIBERI MAKAN MIKROALGA YANG BERBEZA

Oleh

Norfakhrina binti Mohd Noor

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Perhatian terhadap ternakan tiram meningkat secara global. Di Malaysia, tiram komersil, *Crassostrea iredalei* diterima sebagai spesis yang mempunyai nilai komersil disebabkan rasanya yang manis dan berlemak. Walaubagaimana pun, ternakan tiram masih bergantung kepada benih yang dijalankan dari alam semulajadi. Pengutipan benih hanya boleh dikutip dari alam semulajadi pada waktu dan musim tertentu sahaja. Oleh demikian, pengeluaran benih di hatceri adalah sangat penting untuk membantu meningkatkan pengeluaran tiram. Kajian perlu dijalankan untuk menentukan pertumbuhan dan kadar hidup larva yang dihasilkan di hatceri. Kajian ini menekankan aspek pemakanan tiram di dalam hatceri. Tiga spesis mikroalga iaitu *Isochrysis* sp., *Pavlova* sp. dan *Chaetoceros* sp. digunakan sebagai makanan untuk larva (peringkat-D dan peringkat umbo) dan spat *C. iredalei*.

Pada eksperimen pertama, kesan pemberian tiga spesis mikroalgae ke atas larva peringkat-D dan umbo dikaji. Kajian dijalankan selama enam hari untuk larva peringkat D. Manakala, selama empat belas hari untuk larva peringkat umbo. Penggunaan diet *Isochrysis* sp. menunjukkan perbezaan yang ketara ($p<0.05$) dari segi pertumbuhan larva peringkat-D berbanding dengan penggunaan diet *Chaetoceros* sp.. Larva yang diberi makan *Isochrysis* sp. memberikan nilai panjang cengkerang, pertambahan panjang cengkerang dan tumbesaran (85.45 ± 7.72 μm, 22.07 ± 7.72 μm, 0.05 ± 0.02 μm/hari) yang tinggi.

Manakala, diet gabungan flagellat dan diatom memberi pertumbuhan yang baik untuk larva peringkat umbo. Diet *Pavlova* sp./*Chaetoceros* sp. memberi pertumbuhan yang terbaik ($p<0.05$) berbanding diet *Isochrysis* sp./*Pavlova* sp.. Diet *Pavlova* sp./*Chaetoceros* sp. memberikan nilai yang tinggi untuk panjang cengkerang, pertambahan panjang cengkerang dan tumbesaran (265.89 ± 22.50 μm, 184.13 ± 22.50 μm, 0.08 ± 0.01 μm/hari). Kadar hidup larva peringkat D dan larva peringkat umbo dalam setiap diet menunjukkan tiada perbezaan yang ketara ($p>0.05$). Keputusan ini menunjukkan larva peringkat-D memerlukan *Isochrysis* sp. untuk tumbesaran yang baik. Manakala, untuk larva peringkat umbo, diet mengandungi campuran flagellat dan

diatom adalah lebih baik.

Pada eksperimen kedua, kesan pemberian tiga spesis mikroalga keatas spat *C. iredalei* dikaji. Spat diternak selama tiga minggu di dalam bekas 1L. Tiada kematian direkod sepanjang kajian dijalankan keatas spat. Tiada perbezaan yang ketara ($p>0.05$) diantara diet *Isochrysis* sp./*Chaetoceros* sp. (IC) dan *Pavlova* sp./*Chaetoceros* sp. (PC). Kedua-dua diet binari menunjukkan perbezaan yang ketara ($p<0.05$) yang tinggi terhadap diet *Chaetoceros* sp. (C). Diet IC adalah terbaik untuk ukuran akhir, pertambahan panjang cengkerang dan tumbesaran (6.240 ± 0.186 mm, 2.740 ± 0.193 mm, 0.0276 ± 0.002 mm/hari). Diet C menunjukkan nilai terendah untuk ukuran akhir, pertambahan panjang cengkerang dan tumbesaran (5.413 ± 0.486 mm, 1.947 ± 0.365 mm, 0.0211 ± 0.003 mm/hari). Kandungan asid lemak menunjukkan *Isochrysis* sp. kaya dengan kandungan asid dokosaheksanoik (DHA) dan *Pavlova* sp. kaya dengan asid lemak tepu (SFA). Manakala, *Chaetoceros* sp. kaya dengan kandungan lemak tidak tepu (PUFA), asid eikosapentanoik (EPA) dan asid arakidonik (AA). Kekurangan DHA di dalam diet C menyebabkan pertumbuhan spat yang rendah. Jadi, Keputusan ini menunjukkan gabungan diatom dan flagellat sesuai untuk pertumbuhan awal spat *C. iredalei.*

Kesimpulannya, pada peringkat awal larva, *Isochrysis* sp. lebih diperlukan berbanding diatom kerana ianya senang dihadam. Apabila larva membesar ke peringkat seterusnya, ia memerlukan gabungan flagellat dan diatom.

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"*And He it is who has subjected the sea (to you), that you eat thereof fresh tender meat, and that you bring forth out of it ornaments to wear, and you see the ships ploughing through it, that you may seek (thus) of His Bounty (by transporting the goods from place to place) and that you may be grateful*" -AnNahl:14-

I certify that a Thesis Examination Committee has met on December 2, 2016 to conduct the final examination of Norfakhrina binti Mohd Noor on her thesis entitled Growth Performance of Slipper-cupped Oyster, *Crassostrea iredaei* (Faustino, 1932) Larvae and Spat Fed with Different Microalgae in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
BOBP	Bay of Bengal Program
CO_2	Carbon Dioxide
DHA	Docosahexanoic acid
EPA	Eicosapentanoic acid
FAME	Fatty Acid Methyl Esters
FAO	Food of Agriculture Organisation
IDRC	International Development Research Center Canada
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
S.D	Standard Deviation
SFA	Saturated Fatty Acid
^oC	degree Celcius
Hr	Hour
L	Liter
µL	Microliter
Mm	Micrometer
mL	Millilitre
Mm	Millimetre
Ppm	part per million
‰	part per thousand (ppt)
%	Percentage
Pg	Pictogram

CHAPTER 1

INTRODUCTION

1.1 Introduction

Fisheries are divided into capture, aquaculture, ornamental and recreational fisheries (FAO 2016a). According to Van Houtte et al., (1989), aquaculture fisheries are defined as farming of aquatic organism including fish, molluscs, crustacean and aquatic plant. Farming applies some rearing processes to enhances production such as feeding, regular stocking and protection from predators. In recent year, aquaculture becomes popular among farmer. It is one of the food production sector which caters for family, country and global. Aquaculture sector is raising rapidly with increasing demand over the years. Coastal aquaculture is popular in the tropical region. One of the cultured species is mollusks. It represents 14 million tonnes in year 2000 for world aquaculture production. In 2012, the production increased to 15 million tonnes (FAO, 2014).

Culture of mollusks is one of the oldest form of aquaculture existed since Roman times (Sahrhage and Lundbeck, 1992). Oysters from the family Ostreidae are the most common cultured molluscs worldwide with variety including species of *Ostrea* (flat oyster) and *Crassostrea* (cup oyster) being grown. Currently, hatchery production of oysters seed occurs in a large scale in the USA, UK and France. Spawning stock is maintained in a flow through system with high quality saline water with algae added to boost the food supply and temperature manipulation being used to trigger spawning (Frid and Dobson, 2013).

In Malaysia, the tropical oyster, *Crassostrea iredalei* is a potential species for commercial aquaculture because of its sweet flavour and creamy coloured meat (Mohd Yatim, 1993; Devakie and Ali, 2002). These oysters are sold either with shell, half shell or without shell. Oyster is served as food in restaurant and steamboat stall either as ready-made dishes or fresh. Oyster is a nutritious food, easy to digest, rich in minerals and vitamins. They contained low cholesterol as compared to prawn and squids (Reames, 2012).

There are four genera of oysters found in Malaysia which are *Crassostrea iredalei*, *Crassostrea belcheri*, *Ostrea folium*, *Saccostrea cucullata* and *Hyotissa hyotis* (Lovatelli, 1988). In Peninsula Malaysia, oyster are harvested for human consumption. However, oyster culture in Malaysia is still at initial stage due to a considerable number of problems hindering the development commercial culture.

Experiment on nutritional value of various microalgae used as feed for larvae and juveniles have been conducted on different bivalve groups such as clams (Albentosa et al., 1996; Aranda-Burgos et al., 2014), scallops (Milke et al., 2004; Pernet et al., 2005),

sand pearl oyster (Martínez-Fernández et al., 2006; Martínez-Fernández and Southgate, 2007), oysters (Rivero-Rodriguez et al., 2007) and mussels (Pettersen et al., 2010).

Several researches studied the effect of feeding microlagae on the growth performance of oyster (Southgate and Lee, 1998; Doroudi et al., 1999; Doroudi and Southgate, 2000; Martínez-Fernández et al., 2006; Martínez-Fernández and Southgate, 2007; Ronquillo et al., 2012). Microalgae are the major feed for bivalve. Every development stages of bivalve need microalgae as energy source (Ludi, 2011). Species such as *Pavlova salina*, *Chaetoceros* sp., *Skeletonema* sp., and *Micromonas pusilla* have been used as feed for molluscs. These microalgae are fed to molluscs either as a single feed or in combination (Ponis et al., 2003; Martínez-Fernández et al., 2006; Ponis et al., 2006a; Rico-Villa et al., 2006; Sanchez-Lazo, 2014).

Tropical microalgae such as *Chaetoceros* sp., *Pavlova* sp. and *Isochrysis* sp. have been used by many mollusks hatcheries. *Chaetoceros* sp., a diatom with cell wall made up of silica. It looks rigid and brownish in colour. It has setae with long and thin spine (Erlina and Hastuti, 1986). While, *Isochrysis* sp. and *Pavlova* sp. are golden flagellate, ovoid in shape with haptonema. These microalgaeare known to have good nutritional value. Particularly high in protein (Martínez-Fernández et al., 2006).

Microalgae are used as feed for mariculture based on several factors such as cell size, shape, digestibility and biochemical composition (Doroudi et al., 2003, Martínez-Fernández et al, 2004). The species should have high value of either eicosapentanoic acid (EPA, 20:5*n*-3), docosahexanoic acid (DHA, 22:6*n*-3) and other HUFA. To date, very few studies have been conducted using these microalgae as feed for *C. iredalei* (Devakie and Ali, 2000a, 2000b, 2002; Md Saleh et al., 2004; Nor Idayu et al., 2015).

1.2 Problem statements

Production of oyster decreased due to inadequate and inconsistent supply of good quality seed because of most seed supplies are collected from the wild (Kechik, 1995; Tan et al., 2014). The variability of size and age of seed caused difficulty for oyster culturist. Not only that, these seeds are susceptible to mortality. Drastic changed of weather and temperature made it difficult for spat to find the suitable area for settlement. In addition, the excessive industrial and domestic pollutants contribute to the low survival of seed. Water pollution from industries such as oil spills and heavy metal can reduce the quality and quantity of the natural spats. Trace metal of spat can be passed on to the higher trophic level, that is the human race (Forrest et al., 2009). Other factor lead to higher mortality and lower productivity of oyster is ocean acidification. Tropical country like Malaysia receives rain throughout the year which lowered the pH of water. Acidification caused the thinning of the shell therefore making oyster an easy prey (Tan et al., 2014).

Findings on the beneficial values has resulted in high demand for oyster (Martino and Cruz, 2004; Reames, 2012). This circumstance lead to the excessive harvest of oyster for human consumption. Overexploitation of oyster resulted in detrimental effect on the natural bed and reduce its natural breeding ground. Lack of reliable and sufficient supply of oyster spat is a major constraint to commercial oyster culture in Malaysia (Ng, 1979; Wong et al., 1991; Tan et al., 2014). Current production of oysters is still largely dependent on natural spat fall since very few producers have succeeded in hatchery production, due to technological constraint (Ludi, 2011; Frid and Dobson, 2013).

Therefore, hatchery production is important to maintain the seed supply to farmers. Feeding is one of the main factor affecting growth and survival of seed in hatchery. There is little information on the effects of microalgae used as feed for slipper-cupped oyster. Furthermore, there is no established standard on the combination of microalgae for this species.

1.3 Objectives

Objectives of this study were:

i. To determine the effect of mono, binary and ternary feeding using *Isochrysis* sp., *Pavlova* sp., and *Chaetoceros* sp. on the growth of D-stage *C. iredalei* larvae.

ii. To investigate growth and survival of umbo stage larvae of *C. iredalei* fed with mono, binary and ternary combination of *Isochrysis* sp., *Pavlova* sp., and *Chaetoceros* sp..

iii. To determine effect of mono, binary and ternary combination of *Isochrysis* sp., *Pavlova* sp., and *Chaetoceros* sp. on the growth and survival of *C. iredalei* spat.

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