UNIVERSITI PUTRA MALAYSIA

GONAD MATURATION, LARVAL GROWTH AND SETTLEMENT OF THE SLIPPER CUPPED OYSTER Crassostreairedalei FAUSTINO 1932 (MOLLUSCA, PELEYPODA: OSTREIDEI)

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GONAD MATURATION, LARVAL GROWTH AND SETTLEMENT OF THE SLIPPER CUPPED OYSTER *Crassostrea iredalei* FAUSTINO 1932 (MOLLUSCA, PELEYPODA: OSTREIDEI)

By

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GONAD MATURATION, LARVAL GROWTH AND SETTLEMENT OF THE SLIPPER-CUPPED OYSTER *Crassostrea iredalei* FAUSTINO 1932 (MOLLUSCA, PELEYPODA: OSTREIDEI)

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May 2017

Chairman: Aziz Bin Arshad, PhD
Faculty: Agriculture

*Crassostrea iredalei* is a potential oyster species to be cultured in Malaysia intensively. By understanding factors that influence growth and survival of oysters from early development until spat stage will be useful in managing spat production in the hatchery. Therefore, this study has been assessed on gonadal stages, early development of larval, effect of cues on larval settlement, spat growth and capability of tolerance towards certain salinity and temperature. Research was mainly conducted at Fisheries Research Institute (FRI) Pulau Sâyak, Kedah from June 2013 until June 2015. About 140 oysters were determined gonadal stages by biometric measurement and histology. For embryonic and larval development, 280 oysters were stripped-spawning and rearing by normal culture practice. Effects of stocking and algal density on larval development and survival also tested accordingly. Eye-spot larvae were tested for settlement and metamorphosis within 24 and 48 hour by four chemicals, γ-aminobutyric acid (GABA), isobutyl methylxanthin (IBMX), potassium chloride (KCl) and serotonin at different concentrations, microalgae (*Chaetoceros calcitrans, Isochrysis galbana* and *Nannochloropsis* sp.) and marine bacteria. Development of spat were observed and effects of short-term immersion in different salinity (10, 15, 20, 25 and 30 ppt) at fixed temperature 28±1°C and temperatures (15, 19, 23, 27, 31, 35°C) at fixed salinity 29±1 ppt) were determined in relation to growth, survival and clearance rate of spat. Histological results showed four gonadal stages were recognized viz. undifferentiated S1 where no sex cell developed, early development S2 where oocytes initiated, late development S3 where most oocytes developed to vitellogenic and mature S4 where follicles filled with postvitellogenic oocytes. Mature oysters indicated at shell length 73 to 83 mm and weight at 62 to 102 g. Sample of oysters dominated by late development stage S3 at 63 oyster and matured oysters were 31 female and 12 male. Complete planktonic stage of *C. iredalei* was recorded from day 12 to 16 where the early settled larvae was observed. Spatfall began on day 16 onwards. Mean specific growth rate (SGR) from day 2 until day 16 was recorded at 22.16% where growth rate at 14.64 μm day⁻¹ in shell length and 18.60 μm day⁻¹ in shell height. Linear relationship between shell length and shell height of larvae is given by an equation y= 1.2217x - 7.374, $R^2= 0.939$
(n=30). Larval rearing was recommended at 10 larvae mL⁻¹ and algal density between 5000 to 15 000 cells mL⁻¹ for veliger larvae and increase up to 30 000 cells mL⁻¹ in umbo stage. For larval settlement and metamorphosis, highest scored by bacterium identified as *Bacillus cereus* yielded up to 79% at cell density 10⁵ after 48 hour significantly (p<0.05). It is proved that specific cues released by *B. cereus* able to induce high settlement rate within a short period. While only *C. calcitrans* at cell density 10⁵ cells mL⁻¹induced larval settlement at 55% compared to *I. galbana* and *Nannochloropsis* sp. due to presences of eicosapentanoic acid (EPA) and arachidonic acid (ARA) at high level has correlated with the ability of larvae to undergo settlement. All chemicals showed lower larval settlement in all concentration tested at 15% to 17% and only GABA was induced until metamorphosis at 28%. For IBMX and serotonin it is not recommended for *C. iredalei* due to expensive price compared to KCl but all of these chemicals has failed to induce settlement in high percentage within a short period. Larval survival were greater than 50% in all treatments excepted for bacterium C4 and chemicals concentration at 10⁻³ to 10⁻⁴ M onwards. Then, development of pediveliger larvae were observed until spat stage on day 16 onwards. The spats growth measured by shell length increased from day 5 (0.55±0.08 mm), 30 (6.06±0.98 mm) and day 60 (7.91±0.69 mm). Presence of epibiotic polychaete identified under family Spionidae. While effects of short-term immersion of spat at size 6-7 mm showed salinity at 25 ppt resulted high SGR at 18% and clearance rate at 11.14x10³ cells mL⁻¹ ind⁻¹. Temperature at 23°C showed high SGR at 19% and clearance rate at 2.4x10³ cells mL⁻¹ ind⁻¹ which was lower than salinity treatment. It shows that clearance rate affected more on change in temperature compared to salinity where spat tend to close valves as it reduced in filtering algal cells and yet decrease in spat growth. No mortality were observed. This study showed maturated oysters able to be found every month although during rainy season for hatchery spawning. Development of planktonic stage successfully recorded and *C. iredalei* is considered as a fast-grower bivalve. Used of bacteria biofilm identified as *B. cereus* promoted higher settlement and metamorphosis rate on larvae. Spat at size 6-7 mm were recommended for grow-out culture as it able to tolerate at broad range of salinity and temperature.
ABSTRAK TESIS YANG DIKEMUKAN KEPADA SENAT UNIVERSITI PUTRA MALAYSIA SEBAGAI MEMENUHI KEPERLUAN UNTUK IJAZAH DOKTOR FALSAFAH

KEMATANGAN GONAD, TUMBESARAN LARVA AND PENEMPATAN TIRAM TROPIKA Crassostrea iredalei FAUSTINO 1932 (MOLUSKA, PELEYPODA: OSTREIDEI)

OLEH

NOR IDAYU BINTI ABD WAHAB

MEI 2017

Pengerusi: Aziz Bin Arshad, PhD
Fakulti: Pertanian

Ternakan larval dicadangkan pada kepadatan 10 larva mL⁻¹ dan kepadatan alga antara 5000 to 15 000 sel mL⁻¹ bagi veliger dan meningkat sehingga 30 000 sel mL⁻¹ bagi fasa umbu. Bagi penempatan dan metamorphosis larva, skor tertinggi diperolehi oleh bakteria yang dikenali sebagai *Bacillus cereus* sebanyak 70% pada kepadatan sel 10³ selepas 48 jam secara signifikan (*p*<0.05). Ini menunjukkan bahawa petunjuk kimia spesifik yang dibebaskan oleh *B. cereus* berjaya meningkatkan kadar penempatan larva. Manakala hanya *C. calcitrans* pada kepadatan 10⁵ sel mL⁻¹ mengaruhkan penempatan larva sebanyak 55% berbanding *I. galbana* dan *Nannochloropsis* sp. kerana terdapat kandungan asid lemak, asid eikosapentanoik (EPA) dan asid arakidonik (ARA) yang tinggi bagi merangsang larva untuk melekat. Semua bahan kimia ini gagal mengaruhkan kadar penempatan larva lebih rendah sebanyak 15 hingga 17% dan hanya GABA mengaruhkan metamorphosis sebanyak 28%. Bagi IBMX dan serotonin adalah tidak digalakkan penggunaan mereka kerana harga yang mahal berbanding KCl akan tetapi kepadatan bahan kimia ini gagal mengaruhkan penempatan larva yang tinggi dalam tempoh yang singkat. Lebih dari 50% larva berjaya hidup dalam semua rawatan kecuali bagi bakteria C4 dan kepekatan bahan kimia antara 10⁻³ to 10⁻⁴M. Tumbesaran spat diukur dengan peningkatan panjang cengkerang dari hari ke 5 spat (0.55±0.08 mm), 30 (6.06±0.98 mm) dan hari 60 (7.91±0.69 mm). Kehadiran epibiotik poliket dikenali dari keluarga Spionidae. Manakala, kesan rendaman jangka-pendek oleh spat bersaiz 6-7 mm telah menunjukkan saliniti 25 ppt menghasilkan SGR yang tinggi iaitu 18% dan kadar pengurangan alga tertinggi iaitu 11.14x10³ sel mL⁻¹ ind⁻¹ kerana proses penapisan alga untuk dicerminkan oleh spat pada kadar yang maksimum. Suhu pada 23°C menunjukkan SGR tertinggi iaitu 19% dan kadar pengurangan alga sebanyak 2.4x10³ sel mL⁻¹ ind⁻¹ iaitu lebih rendah dari rawatan saliniti. Ini menunjukkan bahawa spat adalah sensitif terhadap perubahan suhu dan cenderung menutup cengkerangnya menyebabkan kuantiti alga yang dapat ditapis berkurang serta akan membantatkan tumbesaran. Tiada kematian direkodkan. Kajian ini telah menunjukkan tiram matang boleh diperolehi setiap bulan walaupun ketika musim hujan untuk peneluran di hatceri. Perkembangan fasa planktonik telah berjaya direkodkan dan *C. iredalei* dianggap sebagai haiwan dwicengkerang bertumbesaran pantas. Penggunaan biofilm bakteria dari *B. cereus* telah meningkatkan penempatan dan metamorphosis larva. Spat bersaiz 6-7 mm disyorkan untuk ternakan luar kerana berkeupayaan hidup pada jual saliniti dan suhu yang tinggi. Saliniti lebih memberi kesan terhadap tumbesaran dan kadar pengurangan alga oleh spat berbanding suhu.
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“And it is He (Allah) who subjected the sea subservient that you may eat from it fresh tender meat, and to bring forth from it ornaments which you wear. And you see the ships sailing through it and (He subjected it) that you may seek of His bounty, and perhaps you will be grateful.”

(An-Nahl 16:14)
I certify that a Thesis Examination Committee has met on 23 May 2017 to conduct the final examination of Nor Idayu binti Abd Wahab on her thesis entitled "Gonad Maturation, Larval Growth and Settlement of the Slipper-Cupped Oyster Crassostrea irideaesi" Faustino 1932 (Mollusca, Peleypoda: Ostreidei)" 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

°C  Degree celcius
ppt  Parts per thousand
cm  Centimeter
kg  Kilogram
μm  Micrometer
μL  Microlitre
%  Percentage
mm  Millimetre
mL  Millilitre
L  Litre
M  Molar
min  Minute
hr  Hour
psi  Pound per square inch
n  number
ANOVA  Analysis of variance
SPSS  Statistic programme for social science
S.D.  Standard deviation
DNA  Deoxyribonucleic acid
CHAPTER 1

INTRODUCTION

1.1 Background of study

Aquaculture is an alternative approach in production of marine seafood as supplying market demand nowadays. Oysters are one potential bivalves for aquaculture and highly contributed to the economic and adaptability to cultivation (Kechik, 1995; Devakie et al., 1993; Angell, 1986). Development of aquaculture registered in 1973 where two species of oyster *Saccostrea cucullata* and *Crassostrea rivularis* found in Peninsular Malaysia and being cultivated by obtained natural spat after monsoon season. Spat were collected by egg-crater filler where it turned out to be the most successful as raft culture method is still under practice (Choo, 1985). In Sarawak, species of oysters found are *C. rivularis*, *C. cucullata* and *C. gigas*, and cultured was experimentally initiated in 1967 by the Inland Fisheries Division of the Department of Agriculture. Then in year 1970, intensive research towards the artificial cultivation of oyster in Sabah were conducted by the State Fisheries Department. The experimental station at Sungai Mapan, Tawau was established in 1973 (Kong and Luh, 1975). In the Muar River, Johor, collection of spat from *C. belcheri* was successfully collected from oyster shells as the most suitable cultch. Application of polyethylene ropes and nets were found to be most effective method for collecting of flat oyster, *Ostrea folium* in Pulau Langkawi (Ng, 1979). Oyster culture in Malaysia has been developed by bottom-culture method in Muar River, Johor, raft-culture method in Pulau Langkawi and rack-culture in Sabah and Sarawak (Ng, 1979).

Department of Fisheries Malaysia under the sponsorship of the Bay of Bengal Programme (BOBP, 1998-1993) commenced the outline of oysters farming in Kedah, Perak, Johor, Kelantan and Terengganu systematically (Devakie et al., 1993). Study on the biology and culture of oysters in the tropics has been supported by the International Development and Research Canada (IDRC) (Tan et al., 2016). Site selection were determined based on availability of oyster seed from wild along the west coast and extended to the east coast of Peninsular Malaysia. Besides, several campaigns has been conducted by the Department of Fisheries Malaysia in promoting of local oysters with collaboration with leading hotels and restaurants from 1993 to 1994 (Devakie et al., 1993).

Production of edible oysters of *C. iredalei* and *C. belcheri* were contributed by Kedah, Pulau Pinang, Perak, Johor, Terengganu and Kelantan. Sabah recorded the highest harvested oysters compared to other states and major on pearl oysters only. Thus, study focusing more on edible oyster must be conducted in reducing reliability of spat from wild only. Previous studies on larval development of local oyster species *C. iredalei* and *C. belcheri* larvae has been established regarding to substrates, storage temperature, salinity-temperature (Devakie and Ali, 2002; 2000b) and chemicals interaction on larval settlement (Teh et al., 2012; Tan and Wong, 1996; 1995). Besides effects of environmental parameters on development and survival of broodstock oyster also being documented (Izwandy, 2006; Hawkins et al., 1998). Used of genetic approaches of
diploid and triploid (Masazurah et al., 2010) and tetraploid induction in oysters, *C. iredalei* and *C. belcheri* also being studied (Tan et al., 2016).

1.2 Problem statement

Studies on *C. iredalei* is still need to explore more as it has high market demand compared to *C. belcheri* in terms of good appearance with whitish flesh. However, grow-out cultivation in Peninsular Malaysia was still relying by transplanting juvenile from its origin, Kelantan and Terengganu to other culture sites including in Kedah, Pulau Pinang and Perak (Danial Hariz et al., 2014; Izwandy, 2006).

However, some main problems affecting oyster culture in Peninsular Malaysia that caused the uncertainty of oyster supply. Mass mortality of oysters (*C. belcheri* and *S. cucullata*) cultured in Sabah were recorded in May 1981 where sudden environmental changes caused due to extensive tree cutting and burning near the culture station. Then, together with heavy rainfall triggered sudden drop of pH and temperature and yet heavy siltation on the site (Ng, 1979). In addition, most of the estuaries and rivers are subjected to heavy siltation and have low densities of phytoplankton as main food to oysters. Sheltered bays are deficient and coastline is subject to strong wave action mainly during monsoons. The excessively high tide range is disadvantage to fisherman where it can destruction the culture systems and caused loss of profit (Devakie et al., 1993; Davenport and Wong, 1992).

Parasites of oysters have been identified in the spat stage during grow-out culture and caused problems during production. Presences of parasites, mudworms of the genus *Polydora* produce a mud tube and blister in oyster shells. Although some of infestations cases did not damage in oyster tissues but the formation of mud blisters affects the appearance, taste and market value of oysters (Menzel, 1990; Bergman et al., 1982). Expanding of oyster cultivation in Malaysia could be much faster if not because of limited seed supply (Devakie et al., 1993; Wong, 1990).

Thus, production of seed from the hatchery can provide the required supply in term of quantity and quality. Many studies are on-going in improving the production of oyster in Malaysia. There were several research has been focused on oyster *C. iredalei* regarding on hybridization of oysters, artificial spawning, environmental factors and hatchery seed production conducted by Universiti Sains Malaysia and Malaysian Government (Tan et al., 2016; Teh et al., 2012; Masazurah et al., 2010; Izwandy, 2006; Devakie and Ali, 2002, 2000a, 2000b). However, there still need more research to be focused on the basic of early development of oyster in terms of duration required and morphological changes in order to sustain data on the originality of larvae development in *C. iredalei* in Malaysia. This knowledge may contribute in increasing hatchery production for continuous spat supply and producing a good quality of juvenile. Hence, to provide an information data on local oyster species for further study.
1.3 Significant of study

There was still less study on gonad histology of *C. iredalei* although prediction of spatfall typically after rainfall season at two peaks in April-June and October-December. Gonad maturity affected the survival and quality of produced larvae. Moreover, the aim of this research is to describe on the early development of *C. iredalei* during meiosis and planktonic larval stage. Although, supported studies has been conducted on larval development influenced by salinity, food and temperatures (Devakie and Ali, 2000a, 2000b; Lau et al., 1992), there is still less information on early development of embryo *C. iredalei* slightly after fertilization.

Hatchery-reared oysters facing a crucial part of larval setting involving settlement and metamorphosis of larvae which may degrade the larval survival. This phase required cues that fasten the processes and only significant cues will induce the setting process. The cue generated from dissolved chemicals, availability of food and space for attachment, bacterial films, adults pheromones and physical properties of water (Grant et al., 2013; Bussarawit and Cedhagen, 2012a; Ganesan et al., 2010; Yu et al., 2010; Bao et al., 2007; Devakie and Ali, 2002; Tan and Wong, 1996; Teh et al., 2012; García-Lavandeira et al., 2005; Dobretsov and Qian, 2003). Thus, settlement and metamorphosis process are the subject to be discovered based on cues that well-studied on the other bivalve species. The characteristics of cues with no detrimental effects on larvae regarding to growth and survival.

Then, early spat growth was tested on its tolerance limits on environmental changes of salinity and temperature. This experiment almost mimicking the environmental conditions in grow-out site at brackish water. Thus, this findings will ensure the optimal age for spat transplant from the hatchery to grow-out site. Consequently, this research is a significant work on biology of oyster *C. iredalei* in Malaysian water as it started with the gonad assessment, describing on larval development and morphological features, cues identification in response to larval settlement and spat growth.

This research was conducted at Fisheries Research Institute (FRI) Pulau Sayak, Kedah started from June 2013 until June 2015. This hatchery has been chosen for research purpose as it well established in good facilities in culturing bivalves including oysters and green mussels (Mohd Saleh, 2011; Masazurah et al., 2010). Moreover, there were ample facilities of culture tanks, source of seawater, algal culture laboratory and expertise person in bivalves. While sample of oysters were collected from local oyster farmer at Sungai Terus, Batu Lintang, Kedah (5°37’16.”N 100°23’19.2”E). This culture site has been identified suitable for oyster culture as it encountered less strong waves and abundant in phytoplankton (Devakie et al., 1993). Sungai Terus is a tributary of Sungai Merbok located along reserved mangroves forest with active fishing activities and fish cage cultures. This location is near to settlement area and active with terrestrial agro-based activities such as shrimp ponds, paddy fields and oil palms. The operation of seafood restaurants in nearby areas increased the amount of freshwater influx into the ecosystem and their waste products are indiscriminately discharged into the estuary area. Oyster farming method mostly by rafting method compared to long-lines.
1.4 Objectives

List of four objectives for this research are highlighted below:

1. To assess the gonadal stages of oyster based on biometric measurements and histology examination

2. To describe the biology of the early embryonic and larval development, effect of stocking densities and algal densities on larval growth and survival of *C. iredalei*;

3. To determine the effects of chemical cues, microalgae and marine bacteria in larval settlement and metamorphosis;

4. To define the early spat growth and effects of short-term immersion of low-high salinities and temperatures on growth and clearance rates of spat *C. iredalei*. 
REFERENCES


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