UNIVERSITI PUTRA MALAYSIA

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

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By

NOR IDAYU BINTI ABD WAHAB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

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

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NOR IDAYU BINTI ABD WAHAB

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 Sayak, 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±1ºC and temperatures (15, 19, 23, 27, 31, 35ºC) 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 5 000 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 10^3 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 10^5 cells mL^{-1} induced larval settlement at 55% compared to *I. galbana* and *Nannochloropsis* sp. due to presences of eicosapentaenoic 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 $11.14x10^3$ cells mL^{-1} ind^{-1}. Temperature at 23°C showed high SGR at 19% and clearance rate at $2.4x10^3$ 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

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Crassostrea iredalei berpotensi sebagai spesies tiram untuk ternakan secara intensif di Malaysia. Dengan memahami faktor-faktor yang mempengaruhi tumbesaran dan kelangsungan hidup tiram dari perkembangan awal sehingga fasa spat adalah penting bagi pengeluaran spat dalam hatceri. Maka, kajian telah dijalankan bagi menentukan fasa gonad, perkembangan awal larva, kesan agen petunjuk terhadap penempatan larva, perkembangan spat dan kebolehan toleransi terhadap pelbagai julat saliniti dan suhu. Kajian telah dijalankan di Institut Penyelidikan Perikanan (FRI) Pulau Sayak, Kedah bermula pada Jun 2013 sehingga Jun 2015. Tiram sebanyak 140 ekor ditentukan fasa gonad dengan pengukuran biometrik dan histologi. Bagi perkembangan embrio dan larva, 280 tiram telah disenyawakan dan diternak seperti amalan ternakan biasa. Kesan kepadatan stok dan alga terhadap perkembangan larva dan kelangsungan hidup telah diuji. Kemudian, penempatan dan metamorphosis larva bermata diuji selama 24 jam dan 48 jam oleh empat bahan kimia iaitu asid γ-aminobutirik (GABA), isobutil methilzanthin (IBMX), kalsium klorida (KCl) and serotonin pada kepekatan berlainan, mikroalga (*Chaetoceros calcitrans, Isochrysis galbana* and *Nannochloropsis* sp.) dan bakteria marin. Perkembangan spat telah dinilai dan kesan rendaman jangka-pendek bagi saliniti berbeza (10, 15, 20, 25 and 30 ppt) pada suhu 28±1^{o}C dan suhu berbeza (15, 19, 23, 27, 31, 35^{o}C) pada saliniti 29±1 ppt telah ditentukan berdasarkan tumbesaran, hidup dan kadar pengurangan alga. Hasil histologi menunjukkan empat fasa gonad telah dikenalpasti iaitu tiada perbezaan S1 di mana tiada jantina sel, perkembangan awal S2 sebagai permulaan perkembangan oosit, perkembangan akhir S3 di mana oosit berkembang ke vitelogenik dan matang S4 menghasilkan oosit postvitelogenik yang memenuhi folikel. Tiram matang ditentukan dengan panjang cengkerang 73 to 83 mm dan berat 62 to 102 g. Sampel tiram didominasi oleh S3 sebanyak 63 tiram dan tiram matang sebanyak 31 betina dan 12 jantan. Fasa planktonik lengkap telah direkodkan dari hari ke 12 sehingga 16 di mana larva terawal melekat telah mula dilihat. Spat terbentuk pada hari ke 16 dan seterusnya. Purata kadar tumbesaran spesifik (SGR) telah direkodkan sebanyak 22.16% di mana kadar tumbesaran panjang cengkerang adalah 14.64 μm hari^{-1} and 18.60 μm hari^{-1} bagi ketinggian cengkerang. Pertalian linear antara panjang dan tinggi cengkerang berdasarkan persamaan y= 1.2217x - 7.374, R^2= 0.939 (n= 30).

Ternakan larval dicadangkan pada kepadatan 10 larva mL^{-1} dan kepadatan alga antara 5 000 to 15 000 sel mL^{-1} bagi veliger dan meningkat sehingga 30 000 sel mL^{-1} bagi fasa umbo. Bagi penempatan dan metamorphosis larva, skor tertinggi diperolehi oleh bakteria yang dikenalpasti sebagai *Bacillus cereus* sebanyak 70% pada kepadatan sel 10^3 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 10^5 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 mengaruhkan kadar penempatan larva lebih rendah sebanyak 15 hingga 17% dan hanya GABA mengaruhkan metamorphosis sebanyak 28%. Bagi IBMX dan serotonin adalah tidak digalakkan pengunaanya bagi *C. iredalei* kerana harga yang mahal berbanding KCl akan tetapi kesemua 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^{-4} M. 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 dikenalpasti 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 $11.14x10^3$ sel mL^{-1} ind^{-1} kerana proses penapisan alga untuk dicernakan oleh spat pada kadar yang maksimum. Suhu pada 23ºC menunjukkan SGR tertinggi iaitu 19% dan kadar pengurangan alga sebanyak $2.4x10^3$ 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 berkurangan serta akan membantutkan 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 dwicengkerangbertumbesaran pantas. Penggunaan biofilem bakteria dari *B. cereus* telah meningkatkan penempatan dan metamorphosis larva. Spat bersaiz 6-7 mm disyorkan untuk ternakan luar kerana berkeupayaan hidup pada julat 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 iredalei* 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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^{o}C	Degree celcius
ppt	Parts per thousand
cm	Centimeter
kg	Kilogram
µm	Micrometer
µL	Microlitre
%	Percentage
mm	Milimetre
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 approaches in production of marine seafood as supplying market demand nowadays. Oysters is 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 turn out to be 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

Acosta-Salmon, H. and Southgate, P. C. 2005. Histological changes in the gonad of the blacklip pearl oyster (*Pinctada margaritifera*). *Mollusc. Res.* 25(2): 71-74.

Agarwal, P. 2014. Pharmaceutical calculations. United States of America. Jones and Bartlett Learning.

Alagarswami, K., Dharmaraj, S., Velayudhan, T. S. Chellam, A., Victor, A. C. C. and Gandhi, A. D. 1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture*. 34: 287-301.

Alfaro, A.C., Young, T. and Ganesan, A.M. 2011. Regulatory effects of mussel (*Aulacomya maoriana* Iredale 1915) larval settlement by neuroactive compounds, amino acids and bacterial biofilms. *Aquaculture*. 322-323: 158-168.

Allen Jr., S.K. and Bushek, D. 1992. Large-scale production of triploid oysters, *Crassostrea virginica* (Gmelin), using "stripped" gametes. *Aquaculture*. 103: 241-251.

Angell, C. L. 1986. The biology and culture of tropical oysters. 1986. International Center for Living Aquaculturetic Resources Management (ICLARM). Manila, Philippines.

Argüello-Guevara, W., Loor, A. and Sonnenholzner, S. 2013. Broodstock conditioning, spawning induction, and early larval development of the tropical rock oyster *Striostrea prismatica* (Gray 1825). *Jour. of Shellf. Res.* 32(3): 665-670.

Asma, M. K., Saberi, K., Hossein, R. and Zohreh, H. Z. 2014. Seaasonal histological comparison of gonad and gametogenesis in female pearl oyster (*Pinctada radiata*) of the Persian Gulf. *Jour. Of the Persian Gulf*. 2(15): 17-24.

Baker, S.M. and Mann, R. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. *Jour. Exp. Mar. Biol. Ecol*. 181: 239-253.

Baloun, A.J and Morse, D.E. 1984. Ionic control settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). *Biol. Bull*. 167: 124-138.

Bao, W-Y, Yang, J-L., Satuito, C.G. and Kitamura, H. 2007. Larval metamorphosis of the mussel *Mytilus galloprovincialis* in response to *Alteromonas* sp. 1: evidence for two chemical cues?. *Mar. Biol*. 152: 657-666.

Barber, B.J. and Blake, N.J. 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *Jour. Exp. Mar. Biol. Ecol*. 52:121-134.

Barber, B. J. and Blake, N. J. 2006. Reproductive physiology. In *Scallop: biology, ecology and Aquaculturecultureculture*, ed. Shumway, S.E. and Parsons, G.J. pp. 357-416. Netherlands. Elsevier Science Publisher.

Bayne, B.L. 1976. Aspects of reproduction in bivalve molluscs. In Estuarine Process, Uses, stress and adaptation to the estuary, ed Wiley, M. pp. 432-448. New York. Academic Press.

Beiras, R. and Widdows, J. 1995. Induction of metamorphosis in larvae of the oyster *Crassostrea gigas* using neuroactive compounds. *Mar. Biol*. 123: 327-334.

Benigner, P.G., Venoit, A. and Poussart, Y. 1999. Principles of pseudofeces rejection on the bivalve mantle: integration in particle processing. *Mar. Ecol. Prog. Ser.* 178: 259-269.

Bergman, K.M., Elner, R.W. and Risk, M.J., 1982. The influence of *Polydora websteri* borings on the strength of the shell of the sea scallop *Placopecten magelllanicus*. *Can. Jour. Zool*. 60: 2551-2556.

Benson, H. J. 2002. Microbiological Applications 8th Edition, Bacterial Population Counts. McGraw Hill. New York.

Blake, J.M. and Evans, J.W. 1972. *Polydora* and other related genera as borers in mollusc shells and other calcerous substrates. *Veliger*. 15: 235-249.

Bonar, D.B., Coon, S.L., Walch, M., Weiner, R.M. and Fitt, W. 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. *Bull. of Mar. Sc.* 46(2): 484-498.

Brackman, G., Defoirdt, T., Miyamoto, C., Van Calenbergh, S., Bossier, P., Nelis, H.J. and Coenye, T. 2008. Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in *Vibrio spp*. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. *BMC Microbiol*. 8:149.

Breese, W.P. and Robinson, A. 1981. Razor clams, *Siliqua patula* (Dixon): Gonadal development, induced spawning and larval rearing. *Aquaculture*. 22: 27-33.

Brown, M.R., Jeffrey, S.W., Volkman, J.K. and Dunstan, G.A. 1997. Nutritional properties of microalgae for mariculture. *Aquaculture*. 151: 315-331.

Brown, M.R., Jeffrey, S.W. And Garland, C.D. 1989. Nutritional aspects of microalgae used in mariculture; a literature review. CSIRO Marine Laboratory Report Number 205. 44pp.

Bussarawit, S. and Cedhagen, T. 2012a. Larvae of the commercial tropical oyster *Crassostrea belcheri* (Sowerby) are induced to settle by pheromones from the adults. *The Thailand Nat. History Mus. Jour.* 6(2): 75-87.

Bussarawit, S. and Cedhagen, T. 2012b. Larvae of commercial and other oyster species in Thailand (Andaman Sea and Gulf of Thailand). *Steenstrupia*. 32(2): 95-162.

Capo, T.R., Bardales, A.T., Gillette, P.R., Lara, M.R., Schmale, M.C. and Serafy, J.E. 2009. Larval growth, development, and survival of laboratory-reared *Aplysia carlifornica*: Effects of diet and veliger density. *Comp. Biochem. and Physiol., Part C*. 149: 215-223.

Carriker, M.R. 1967. Ecology of estuarine benthic invertebrates: a perspective. Publication of the American Association for the Advancement of Science. 83: 442-487.

Chanley, P.E. and Andrew, J.D. 1971. Aids for the identification of bivalve larvae of Virginica. *Malacol*. 21: 45-120.

Chavez-Villalba, J., Cochard, J.C., Le Pennec, M., Barret, J., Enriquez-Diaz, M. and Caceres-Martinez, C. 2003. Effects of temperature and feeding regimes on gametogenesis and larval production in the oyster *Crassostrea gigas. Jour. of Shellf. Res*. 22:721-731.

Chen, J-Y., Bottjer, D.J., Davidson, E.H., Dornbos, S.Q., Gao, X., Yang, Y-H., Li, C-W., Li, G., Wang, X-Q., Xian, D-C.,Wu, H-J., Hwu, Y-K., Tafforeau, P. 2006. Phosphatized polar-lobe-forming embryos from the Precambrian of Southwest China. *Sci*. 312 (5780): 1644-1646.

Chin, P.K. and Lim, A.L. 1975. Some aspects of oyster culture in Sabah, Kuala Lumpur, Malaysia. Ministry of Agriculture and Rural Development. Fisheries Bulletin. 5, 14pp.

Choi, Y.H. and Chang, Y.J. 2003. Gametogenic cycle of the transplanted culture pearl oyster, *Pinctada fucata martensi* (Bivalvia, Pteridae) in Korea. *Aquaculture*. 220: 781-790.

Christophersen, G. and Strand, O. 2003. Effect of reduced salinity on the great scallop (*Pecten maximus*) spat at two rearing temperatures. *Aquaculture*. 215: 79-92.

Choo, P.S. 1985. Oyster culture. Fisheries Research Institute Glugor, Penang, Malaysia.

Cole, H.A. and Knight-Jones, E.W. 1949. The setting behaviour of larvae of the European flat oyster *Ostrea edulis* L., and its influence on methods of cultivation and spat collection. *Fishery Investigations, Series 2.* 17(3):1-39.

Coon, S.L., Fitt, W.K. and Bonar, D.B. 1990. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas. Mar. Biol*. 106: 379-387.

Coon, S.L., Bonar, D.B. and Weiner, R.M., 1985. Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg), by L-DOPA and catecholamines. *Jour. of Exp. Mar. Biol. and Ecol.* 94: 211-221.

Costerton, J.W. 1999. Introduction to biofilm. *Inter. Jour. of Antimicrob. Agents*. 11: 217-221.

Da Costa, F., Darriba, S. and Martínez-Patiño, D. 2008. Embryonic and larval development of *Ensis arcuatus* (Jeffreys, 1865) (Bivalvia: Pharidae). *Jour. of Mollus. Stud*. 74: 103-109.

Danial Hariz, Z.A., Suzana, M.m Masazurah, A.R., Devakie, M.N., Darlina, M.N. and Siti Azizah, M.N. 2014. Population genetics of the black scar oyster, *Crassostrea iredalei*: repercussion of anthropogenic interference. *Mitochondrial DNA*. 1-12.

Davenport, J. and Wong, T. M. 1992. Effects of temperature and aerial exposure on the three tropical oyster species, *Crassostrea belcheri, Crassostrea iredalei* and *Saccostrea cucullata*. *Jour. Therm. Biol*. 17(3): 135-139.

De Gregoris, T.B. Khandeparker, L., Anil, A.C., Mesbahi, E., Burgess, J.G. and Clare, A.S. 2012. Characterisation of the bacteria assosciated with barnacle, *Balanus amphitrite*, shell and their role in gregarious settlement of cypris larvae. *Jour. of Exp. Mar. Biol. and Ecol*. 413: 7-12.

Department of Fisheries. http://www.dof.gov.my/fishery-statistics online 6 June 2016.

Devakie, M. N. And Ali, A. B. 2002. Effective use of plastic sheet as substrate in enhancing tropical oyster (*Crassostrea iredalei* Faustino) larvae settlement in the hatchery. *Aquaculture*. 212: 277-287.

Devakie, M. N. and Ali, A. B. 2000a. Effects of storage temperature and duration on the setting and post-set spat survival of the tropical oyster, *Crassostrea iredalei* (Faustino). *Aquaculture.* 190 (3-4): 369-376.

Devakie, M.N. and Ali. 2000b. Salinity-temperature and nutritional effects on the setting rate of larvae of the tropical oyster, *Crassostrea iredalei* (Faustino). *Aquaculture*. 184: 105-114.

Devakie, N., Hall, R. and Angell, C.L. 1993. Small-scale oyster culture on the west coast of Peninsular Malaysia: Bay of Bengal Programme. Nagaraj and Co. Madras, India.

Dharmaraj, S., Shanmugasundaram, K. and Suja, C. P. 2004. Larval rearing and spat production of the windowpane shell *Placuna placenta*. *Aquacultureculture Asia*, April-June. 9(2): 20-23.

Dinamani, P. 1976. The morphology of the larval shell of *Saccostrea glomerata* (Gould, 1850) and a comparative study of the larval shell in the genus *Crassostrea* Sacco, 1879 (Ostreidae). *Jour. of Mollusc. Stud*. 42: 95-107

Dobretsov, S.V. and Qian, P-Y. 2003. Pharmacological induction of larval settlement and metamorphosis in the blue mussel *Mytilus edulis* L. *Biofoul*. 19(1): 57-63.

Doroudi, M.S. and Southgate, P.C. 2002. The effect of chemical cues on settlement behavior of blacklip pearl oyster (*Pinctada margaritifera*) larvae. *Aquaculture*. 209: 117-124.

Doroudi, M.S., Southgate, P.C. and Mayer, R.J. 1999. Growth and survival of blacklip pearl oyster larvae fed different densities of microalgae. *Aquaculture. Int*. 7: 179-187.

Dunham, A. and Marshall, R.D. 2012. Using stocking density modifications and novel growth medium to control shell deformities and biofouling in suspended culture of bivalves. *Aquaculture*. 324-325: 234-241.

Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J. and Le Pennec, M. 2009. Gametogenesis, reproductive investment and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy. *Aquaculture. Int.* 17(5): 491-506.

Ferreira, M. A. P., Paixão, L. F., Alcântara-Neto, C. P., Santos, S. S. D. and Rocha, R. M. 2006. Morphological and morphometric aspects of *Crassostrea rhizophore* (Guilding, 1828) oocytes in three stages of the gonadal cycle. *Int. Jour. Morphol.* 24(3): 437-442.

Fearmann J. and Moltschaniwskyj, N. A. 2010. Warmer temperature reduce rates of gametogenesis in temperate mussels, *Mytilus galloprovincialis*. *Aquaculture*. 305: 20-25.

Fitt, W.K., Coon, S.L., Walch, M., Weiner, R.M., Colwell, R.R. and Bonar, D.B. 1990. Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Mar. Biol*. 106: 389-394.

Food and Agriculture Organization of the United Nations (FAO). Retrived 19 May 2016 from http://www.fao.org/fishery/species/2673/en

Fournier, M. L. 1992. The reproductive biology of the tropical rocky oyster *Ostrea iridescens* (Bivalvia: Ostreidae) on the Pacific coast of Costa Rica. *Aquaculture*. 101: 371-378.

Fournier, J., Levesque, E., Pouvreau, S., Le Pennec, M. and Le Moullac, G. 2012. Influence of plankton concentration on gametogenesis and spawning of the black lip pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon (Tuamotu archipelago, French polynesia). *Mar. Poll. Bull*. 65: 463-470.

Gallager, S.M. and Mann, R. 1981. Use of lipid staining techniques for assaying condition in cultured bivalve larvae. *Jour. Shellf. Res*. 1: 69-73.

Ganesan, A.M., Alfaro, A.C., Higgins, C.M., Duxbury, M. and Brooks, J.D. 2012. Characterization of biofilms exudates and their effects on the settlement of mussel (*Perna canaliculus*) larvae. *Jour. of Exp. Mar. Biol. and Ecol*. 434-435: 34-46.

Ganesan, A. M., Alfaro, A. C., Brooks, J.D. and Higgins, C.M. 2010. The role of bacterial biofilms and exudates on the settlement of mussel (*Perna canaliculus*) larvae. *Aquaculture*. 306: 388–392.

García-Lavandeira, M., Silva, A., Abad, M., Pazos, A.J. Sánchez, J.L. and Pérez-Parallé, L. 2005. Effects of GABA and epinephrine on the settlement and metamorphosis of the larvae of four species of bivalve molluscs. *Jour. of Exp. Mar. Biol and Ecol.* 316:149-159.

Grant, M.N., Meritt, D. W. and Kimmel, D.G. 2013. Chemical induction of settlement behavior in larvae of the eastern oyster *Crassostrea virginica* (Gmelin). *Aquaculture.* 402-403: 84-91.

Guimarey, P., Darigran, G., Damborenea, C. and Penchaszadeh, P. E. 2011. Assessment of gonadal follicle size in the invading bivalve *Limnoperna fortunei* (Mytilidae). *Biocell*. 35(2): 59-62.

Hadi, H., Mohd Hanafi, I., Amy Halimah, R. and Abu Hena, M.K. 2015. Reproductive cycle of hard clam, *Meretrix iyrata* Sowerby, 1851 (Bivalvia: Veneridae) from Sarawak, Malaysia. *Trop. Life Sci. Res*. 26(2): 59-72.

Handley, S.J. and Bergquist, P.R. 1997. Spionid polychaete infestations of intertidal pacific oysters *Crassostrea gigas* (Thunberg), Mahurangi Harbour, northern New Zealand. *Aquaculture*. 153: 191-205.

Haris, V. A. 1990. Shell. In *Sessile animals of the sea shore*, pp. 91. London: Chapman and Hall.

Hawkins, A.J.S., Smith, R.F.M., Tan, S.H. and Yasin, Z. B. 1998. Suspension-feeding behaviour in tropical bivalve molluscs: *Perna viridis, Crassostrea belcheri, Crassostrea iredalei, Saccostrea cucclata* and *Pinctada margaritifera. Mar. Ecol. Prog. Ser.* 166: 173-185.

Hawkins, A. J. S. And Bayne, B. L. 1992. Physiological processes and the regulation of production. In *The mussel Mytilus: ecology, physiology, genetics and culture*, ed. E. Gosling, pp.171-222. Amsterdam: Elsevier Science Publisher BV.

Henderson, B.A.1983. Handling and Remote Setting Techniques for the Pacific oyster larvae, *Crassostrea gigas*. Master Thesis. Oregon State University. 37 pp.

Hodgson, C. A. and Burke, R. D. 1988. Development and larval morphology of the spiny scallop, *Chlamys hastata. Biol. Bull*. 174: 303-318.

Holliday, J.E., Allan, G.L. and Nell, J. A. 1993. Effects of stokcing density on the juvenile Sydney rock oysterd, *Saccostrea commercialis* (Iredalei and Roughley), in cylinders. *Aquaculture*. 109: 13-26.

Holliday, J.E., Maguire, G.B. and Nell, J.A. 19991. Optimum stocking density for nursery culture of Sydney rock oysters (*Saccostrea commercialis*). *Aquaculture*. 96: 7-16.

Honkoop, P.J.C. and Bayne, B.L. 2002. Stocking density and growth of the Pacific oyster (*Crassostrea gigas*) and the Sydney rock oyster (*Saccostrea glomerata*) in Port Stephens, Australia. *Aquaculture*. 213: 171-186.

Huang, S. and Hadfield, M.G. 2003. Composition and density of bacterial biofilms determine larval settlement of the polychaete *Hydroides elegans*. *Mar. Ecol. Prog. Ser.* 260: 161-172.

Huo, Z., Wang, Z., Liang, J., Zhang, Y., Shen, J., Yao, T., Su, J. and Yu, R. 2014. Effects of salinity an embryonic development, survival and growth of *Crassostrea hongkongensis. Jour. Ocean Univ. China.* 13 (4): 666-670.

Ian, D. 1991. Chapter Six: Oysters in Shellfish: A guide to oysters, mussels, scallops, clams and similar products for the commercial user, pg 69-109. Van Nostrand Reinhold, New York.

Ir. Mulyanto. 1987. Teknik budidaya laut tiram mutiara di Indonesia. Jaringan Informasi Perikanan Indonesia. Direktorat Jenderal Perikanan. Jakarta.

Izwandy, B. I. 2006. *Pengaruh faktor-faktor persekitaran terhadap pertumbuhan dan kemandirian tiram komersil, Crassostrea iredalei (Faustino) di kawasan penternakan tiram di Kg. Telaga Nenas, Perak*, Master Thesis, Universiti Sains Malaysia.

Jagadis, I. 2011. Spawning, larval development and spat settlement in the Venus clam *Gafrarium tumidum* (Roding, 1798) from south-east coast of India. *Indian Jour. Fish*. 58 (2): 1-5.

Jefferson, K.K. 2004. What drives bacteria to produce a biofilm?. *FEM Microb. Lett*. 236: 163-173.

Juliette, R., Catherine, S., Kyung-II, P., Pouvreau, S., Choi, K. S. and Katherine, C. 2008. Gametogenetic cycle and reproductive effort assessed by two methods in 3 age classes of Pacific oysters, *Crassostrea gigas*, reared in Normandy. *Aquaculture*. 277: 313-320.

Juliette, R., Ropert, M., Mathieu, M. and Costil, K. 2006. Presence of spionid worms and other epibionts in Pacific oysters (*Crassostrea gigas*) cultured in Normandy, France. *Aquaculture*. 253: 461-474.

Kechik, I. A. 1995. *Aquaculture* in Malaysia. Towards sustainable *Aquaculture* in Southeast Asia and Japan. Proceedings of the Seminar–Workshop on

Aquaculture Development in Southeast Asia, Held 26-28 July 1994 in Iloilo City, Philippines. SEAFDEC. *Aquaculture* Department, Iloilo, Philippines, pp. 125-135.

Ke, C.H., Li, S.J., Li, F.X. and Zheng, Z. 1998. Ionic control of attachment and metamorphosis in the green mussel *Perna viridis* (Linnaeus) larvae. *Oceanol. and Limnol. Sin*. 29: 128-134.

Khandeparker, L., Anil, A.C. and Raghukumar, S. 2006. Relevance of biofilm bacteria in modulating the larval metamorphosis of *Balanus amphitrite. FEMS Microbiol. Ecol.* 58: 425-438.

Knight-Jones, E.W. 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *Jour. Exp. Biol.* 30: 584-599.

Kong, C.P. and Luh, L.A. 1975. Some aspects of oyster culture in Sabah. Fisheries. Timbalan Ketua Pengarah Percetakan. Kuala Lumpur.

Laing, I. 2002. Effect of salinity on growth and survival of king scallop spat (*Pecten maximus*). *Aquaculture*. 171-181.

Laing, I. and Psimopoulous, A. 1998. Hatchery cultivation of king scallop (*Pecten maximus*) juveniles with cultured and bloomed algae diets. *Aquaculture*. 169: 55-68.

Lango-Reynoso, F., Chavez-Villalba, J., Cochard, J. C. and Le Pennec, M. 2000. Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster*, Crassostrea gigas* Thunberg. *Aquaculture*. 190: 183-199.

Lau, S.C.K., Mak, K.K.W., Chen, F. and Qian, P-Y. 2002. Bioactivity of bacterial strains isolated from marine biofilms in Hong Kong waters for the induction of larval settlement in the marine polychaete *Hydroides elegans. Mar. Ecol. Prog. Ser.* 226: 301-310.

Lau, K. T., Wong, T. M. and Tan, S. H. 1992. Effect of salinity and feed concentration on larval growth and survival in the tropical oyster, *Crassostrea iredalei* (Faustino). 3rd Asian Fisheries Forum, Singapore, 26-30 Nov. 1992. Asian Fisheries Society, Manila, Philippines.

Laxmilatha, P., Rao, G.S., Patnaik, P., Nageshwara Rao, T., Prasad Rao, M. and Dash, B. 2011. Potential for the hatchery production of spat of the green mussel *Perna viridis* Linnaeus (1758). *Aquaculture.* 312: 88-94.

Leise, E.M., Thavaradhara, K., Durham, N.R. and Turner, B.E. 2001. Serotonin and nitric oxide regulate metamorphosis in the marine snail *Ilyanassa obsoleta*. *Amer. Zool*. 41: 258-267.

Liang, Z. L., Kim, Y. H., Zhang, Z. F., Lim, S. M. and Kang, K. H. 2009. Water temperature and salinity tolerance of embryos and spat of the mussel, *Musculista senhousia. Korean Jour. Malacol*. 25 (3): 179-187.

Lim, P-E., Lee, C-K. and Din, Z. 1995. Accumulation of heavy metals by cultured oysters from Merbok estuary, Malaysia. *Mar. Poll. Bull*. 31 (4-12): 420-423.

Liu, M., Gurney-Smith, H., Beerens, A. and Pearce, C.M. 2010. Effects of stocking density, algal density, and temperature on growth and survival of larvae of the basket cockle, *Clinocardium nuttallii. Aquaculture*. 299: 99-105.

Liu, B., Dong, B., Tang, B., Zhang, T. and Xiang, J. 2006. Effect of stocking density on growth, settlement and survival of clam larvae, *Meretrix meretrix*. *Aquaculture*. 258: 344-349.

Loor, A. and Sonnenholzner, S. 2014. Reproductive cycle of the rock oyster, *Striostra prismatica* (Gray, 1825) from two locations on the southern coast of Ecuador. *Aquaculture. Res*. 1-11.

Loosanoff, V. and Davis, H.C. 1963. Rearing of bivalve larvae. *Adv. in Mar. Biol*. 1:1-136.

Loosanoff, V.L. 1949. On the food selectivity of oysters. *Sci*. 110: 122.
Lowe, D. M., Moore, M. N. and Bayne, B. L. 1982. Aspects of gametogenesis in the marine mussel *Mytilus edulis L. Jour. Mar. Biol. Assoc. U.K*. 62: 133-145.
Machensen, A. K., Brey, T. and Sonnenholzner, S. 2011. The after of *Spondylus* stocks (Bivalvia: Spondylidae) in Ecuador: is recovery likely?. *Jour. of Shellf. Res*. 30: 115-121.
Madrones-Ladja, J.A. 2002. Salinity effect on the embryonic development, larval growth and survival at metamorphosis of *Placuna placenta* Linnaeus (1758). *Aquaculture*. 214: 411-418.
Madrones-Ladja, J.A. 1997. Notes on the induced spawning, embryonic and larval development of the window-pane shell, *Placuna placenta* (Linnaeus, 1758), in the laboratory. *Aquaculture*. 157: 137-146.
Maguire, J.A. and Burnell, G. M. 2011. The effect of stocking density in suspended culture on growth and carbohydrate content of the addudctor muscle in two populations of scallop (*Pecten maximus* L.) in Bantry Bay, Ireland. *Aquaculture*. 198: 95-108.
Malaysian Meteorogical Department (MMD). Retrived 14 October 2015 from http://www.met.gov.my/index.php?option=com_contentandtask=viewandid=844andItemid=1586/
Mann, R., Melissa, S., Carnegie, R. B. and Crockett, R. K. 2014. Temporal variation in fecundity and spawning in the Eastern oyster, *Crassostrea virginica*, in the Piankatank River, Virginia. *Jour. Of Shellf. Res*. 33 (1): 167-176.
Manoj, N and Appukuttan, K. K. 2003. Effect of temperature on the development, growth, survival and settlement of green mussel *Perna viridis* (Linnaeus, 1758). *Aquaculture. Res*. 34: 1037-1045.
Masazurah, A.R., Mohd Saleh, M.T. and Devakie, M.N. 2010. Performance of mass produced diploid vs triploid tropical oyster, *Crassostrea iredalei* Faustino. *Malay. Fish. Jour.* 9: 15-21.
McFarland, K., Donaghy, L. and Volety, A. K. 2013. Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, and the native oyster, *Crassostrea virginica*, in Southwest Florida. *Aquaculturet. Inv*. 8 (3): 299-310.
Menzel, W. 1990. Estuarine and marine bivalve mollusk culture. Boca Raton, Florida, CRC Press, Inc.
Mesías-Gansbiller, C., Silva, A., Maneiro, V., Pazos, A. Sánchez, J.L. and Pérez-Parallé, M.L. 2013. Effects of chemical cues on larval settlement of the flat oyster (*Ostrea edulis* L.): A hatchery approach. *Aquaculture*. 376-379: 85-89.
Mohd Saleh, M.T. 2011. Hatchery production performance of the green mussel, *Perna viridis* (Linnaeus, 1758). *Malay. Fish. Jour*. 10 (1): 1-11.
Mohamad Yatim, H. N. 1995. A guide to oyster culture in Malaysia. Bay of Bengal Programme (BOBP/ MAG/ 18).
Mouëza, M., Gros, O. and Frenkiel, L. 1999. Embryonic, larval and postlarval development of the tropical clam, *Anomalocardia brasiliana* (*Bivalvia, Veneridae*). *Jour. of Mollusc. Stud*. 65: 73-88.
Nell, J.A. and Holliday, J.E. 1988. Effects of salinity on the growth and survival of Sydney rock oyster (*Saccostrea commercialis*) and Pacific oyster (*Crassostrea gigas*) larvae and spat. *Aquaculture*. 68: 39-44.
Ng. F.O. 1979. Experimental culture of a flat oyster (*Ostrea folium* L.) in Malaysian waters. *Malay. Agri. Jour*. 52: 2.
Nor Idayu, A.W. 2011. Kajian perkembangan dan aruhan proses penempatan larva kupang *Perna viridis*, Master Thesis, Universiti Kebangsaan Malaysia.

Nur Leena, W. and Aziz, A. 2013. Induced spawning and early development of *Modiolus philippinarum* (Hanley, 1843) (Bivalvia: Mytilidae). *Asian Jour. of Ani. and Vet. Adv*. 8 (1): 100-107.

O'Connor, W.A. and Heasman, M.P. 1995. Spawning induction and fertilisation in the doughboy scallop *Chlamys* (*Mimachlamys*) *asperrima. Aquaculture*. 136: 117-129.

O'Connor, W. A. and Lawler, N.F. 2005. Emersion tolerance of pearl oyster, *Pinctada imbricata* Röding, spat and juveniles. *Asian Fish. Sc*. 18: 217-224.

O'Toole, G.A. 2011. Microtiter formation assay. *Jour. Of Visual. Exp*. 47. URL: http://www.jove.com/details.php?id=2437

Paixão, L., Ferreira, M. A., Nunes, Z., Fonseca-Sizo, F. and Rocha, R. 2013. Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (*Crassostrea gasar*): Implications for the collection of broodstock oysters. *Aquaculture*. 380-383: 6-12.

Parsons, G.J., Dadswell, M.J. and Roff, J.C. 1993. Influence of biofilm on settlement of sea scallop, *Placopecten magellanicus* (Gmelin, 1791) in Passamaquoddy Bay, News Brunswick, Canada. *Jour. Shellf. Res*. 12: 279-283.

Pawlik, J.R. and Hadfield, M.G. 1990. A symposium on chemical factors that influence the settlement and metamorphosis of marine invertebrate larvae: introduction and perspective. *Bull. Mar. Sci*. 46: 450-454.

Pechenik, J.A., Eyster, L.S., Widdows, J. and Bayne, B.L. 1990. The influence of food concentration and temperature on growth and morphology differentiation of blue mussel *Mytilus edulis* L. larvae. *Jour. Exp. Mar. Biol. Ecol*. 136: 47-64.

Pettersen, A.K., Turchini, G.M., Samad, J., Ingram, B.A. and Sherman, C.D.H. 2010. Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus galloprovincialis*) larvae. *Aquaculture*. 309: 115-124.

Ponis, E., Robert, R. and Parisi, G. 2003. Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). *Aquaculture*. 221: 491-505.

Pouvreau, S., Gangnery, A., Tiapari, J., Lagarde, F., Garnier, M. and Bodoy, A. 2000. Gametogenic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atoll (French Polynesia). *Aquaculturet. Living Resour*. 13 (1): 37-48.

Raghavan, G. and Gopinathan, C.P. 2008. Effects of diet, stocking density and environmental factors on growth, survival and metamorphosis of clam, *Paphia malabarica* (Chemnitz) larvae. *Aquaculture. Res*. 39: 928-933.

Rees, C.B. 1950. The identification and classification of Lamellibranch larvae. *Hull Bull. of Mar. Ecol*. 3: 73-104.

Rico-Villa, B., Pouvreau, S. and Robert, R. 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. *Aquaculture.* 287: 395-401.

Rico-Villa, B., Le Coz, J.R. and Robert, M.R. 2006. Influence of phytoplankton diet mixtures on microalgae consumption, larval development and settlement of the Pacific oyster *Crassostrea gigas* (Thunberg). *Aquaculture*. 256: 377-388.

Roberts, R.D. and Lapworth, C. 2001. Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. *Jour. of Exp. Mar. Biol. and Ecol*. 258: 1-13.

Rodríguez-Jaramillo, C., Hurtado, M. A., Romero-vivas, E., Ramírez, J. L., Manzano. M. and Palcios, E. 2008. Gonadal development and histochemistry of the

tropical oyster, *Crassostrea corteziensis* (Hertlein, 1951) during an annual reproductive cycle. *Jour. of Shellf. Res*. 27 (5): 1129-1141.

Rosell, N. C. 1991. The slipper-shaped oyster (*Crassostrea iredalei*) in the Philippines. In estuarine and marine bivalve mollusk ed. Winston Menzel. 307-13. Boca Raton, Florida. CRC Press.

Sánchez-Lazo, C. and Martínez-Pita, I. 2014. Effects of different mono, bi and trispecific microalgal diets on survival, growth, development, settlement and fatty acid composition of mussel *Mytilus galloprovincialis* (Lamarck, 1819) larvae. *Aquaculture*. 426-427: 138-147.

Sánchez-Lazo, C. and Martínez-Pita, I. 2012. Induction of settlement in larvae of the mussel *Mytilus galloprovincialis* using neuroactive compounds. *Aquaculture*. 344-349: 210-215.

Sahavacharin, S. 1987. Methodology of induced spawning of oyster. Department of Fisheries, Bangkok, Thailand. Technical Paper, Contribution 2, 11pp.

Shumway, S. E., Cucci, T. L., Newell, R. C. and Yentsch, C. M. 1985. Particle selection, ingestion and absorption in filter-feeding bivalves. *Jour. of Exp. Mar. Biol. and Ecol.* 91: 77-92.

Skeel. M.E. 1979. Shell boring worms (Spionidae: Polychaeta) infecting cultivated bivalve mollusc in Australia. *Proc. World of Maric. Soc*. 10: 529-533.

Southgate, P.C. and Lee, P. S. 1998. Hatchery rearing of the tropical blacklip oyster *Saccostrea echinata* (Quoy and Gaimard). *Aquaculture*. 169: 275-281.

Stephen, D. 1980. The reproductive biology of the Indian oyster *Crassostrea madrasensis* (Preston). *Aquaculture*. 21: 139-146.

Sudrajat, A. 1990. Studies on the reproductive biology and culture of the rock oyster, *Saccostrea cucullata* (Born) and slipper oyster, *Crassostrea iredalei* (Faustino). Ph.D Thesis, University College of Swansea.

Suja, N. and Muthiah, P. 2009. Effect of starvation and temperature on gonad development of baby clam, *Marcia opine* (Gmelin). *Jour. Mar. Biol. Ass. India*. 51(1): 21-25.

Suquet, M., Amourda,C., Mingant, C., Quéau, I., Lebrun, L. and Brizard, R. 2007. Setting of a procedure for experimental inducation of Pacific oyster (*Crassostrea gigas*) embryos. *Aquaculture*. 273: 503-508.

Takami, H., Kawamura, T. and Yamashita, Y. 2002. Effects of delayed metamorphosis on larval competence and postlarval survival and growth of abalone *Haliotis discus hannai*. *Aquaculture*. 213: 311-322.

Tamayo, D., Ibarrola, I. and Navarro, E. 2013. Thermal dependence of clearance rate and metabolic rates in slow- and fast-growing spats of manila clam *Ruditapes pilippinarium*. *Jour. Comp. Physiol. B*. 183: 893-904.

Tamburri, M.N., Luckenbach, M.W., Breitburg, D.L. and Bonniwell, S.M. 2008. Settlement of *Crassostrea ariakensis* larvae: effects of substrate, biofilms, sediment and adult chemical cues. *Jour. of Shellf. Res*. 27(3): 601-608.

Tan, S.H.A., Teh, C.P., Chang, G. O. and Yasin, Z. 2016. Tetraploid induction in oysters, *Crassostrea belcheri* (Sowerby) and *Crassostrea iredalei* (Faustino). *Aquaculture. Res*. 1-7.

Tan, S. H. 1993. *Aspects on biology and larval development of tropical oyster, Crassostrea belcheri* Sowerby, Master Thesis, Universiti Sains Malaysia.

Tan, S. H and Wong, T. M. 1996. Effect of salinity on hatching, larval growth, survival and settling in the tropical oyster *Crassostrea belcheri* (Sowerby). *Aquaculture*. 145: 129-139.

Tan, S-H and Wong, T-W. 1995. Induction of settlement and metamorphosis in the tropical oyster, *Crassostrea belcheri* (Sowerby), by neuroactive compounds. *Jour. of Shellf. Res*. 14 (2): 435-438.

Tanyaros, S. and Fitt, L.D. 2011. Larval settlement and spat growth of the tropical oyster *Crassostrea belcheri* (Sowerby 1871) in response to substrate preparations. *Asian. Fish. Sc*. 24: 443-452.

Taylor, J.J., Rose, R.A., Southgate, P.C. and Taylor, C.E. 1997a. Effects of stocking density on growth and survival of early juvenile silver-lip pearl oysters, *Pinctada maxima* (Jameson), held in suspended nursery culture. *Aquaculture*. 153: 41-49.

Taylor, J. J., Southgate, P. C., Wing, M. S. and Rose, R. A. 1997b. The nutritional value of five species of microalgae for spat of the silver-lip pearl oyster, *Pinctada maxima* (Jameson) (Mollusca: Pteriidae). *Asian, Fish. Sc*. 10: 1-8.

Teh, C.P., Zulfigar, Y. and Tan, S.H. 2012. Epinephrine and L-DOPA promote larval settlement and metamorphosis of the tropical oyster, *Crassostrea iredalei* (Faustino, 1932): An oyster hatchery perspective. *Aquaculture*. 38-341: 260-263.

Torkildsen, L., Lambert, C., Nylund, A., Magnesen, T. and Bergh, Ø. 2005. Bacteria associated with early life stages of the great scallop, *Pecten maximus*: impact on larval survival. *Aquaculture. Int*. 13: 575-592.

Tritar, S., Prieur, D. and Weiner, R. 1992. Effects of bacterial films on the settlement of the oysters, *Crassostrea gigas* (Thunberg, 1973) and *Ostrea edulis* (Linnaeus, 1750) and the scallop *Pecten maximus* (Linnaeus, 1758). *Jour. Shellf. Res*. 11: 325-330.

Velasco, L.A., Barros, J. and Acosta, E. 2007. Spawning induction and early development of the Caribbean scallops *Agropecten nucleus* and *Nodipecten nodosus. Aquaculture*. 266: 153-165.

Vélez, A., Alifa, E. and Azuaje, O. 1990. Induction of spawning by temperature and serotonin in the hermaphroditic tropical scallop, *Pecten ziczac. Aquaculture*. 84: 307-313.

Ver, L.M.M. 1986. Early development of *Crassostrea iredalei* (Faustino, 1932) (Bivalvia, Ostreidae), with notes on the structure of the larval hinge. *Veliger*. 29: 78-85.

Verween, A., Vincx, M. and Degraer, S. 2007. The effect of temperature and salinity on the survival of *Mytilopsis leucophaeta* larvae (Mollusc, Bivalvia): The research for environmental limits. *Jour. of Exp. Mar. Biol. and Ecol*. 348: 111-120.

Walne, P.R. 1974. Culture of Bivalve Molluscs: 50 Years' Experience at Conway. Fishing News, West Byfleet, 173 pp.

Wassnig, M. and Southgate, P.C. 2012. Effects of settlement cue on behaviour and substrate attachment of hatchery reared winged pearl oyster (*Pteria penguin*) larvae. *Aquaculture*. 344-349: 216-222.

Weiner, R.M., Walch, M., Labare, M.P., Bonar, D.B. and Colwell, R.R. 1989. Effect of biofilms of the marine bacterium *Alteromonas colwelliana* (LST) on set of the oysters *Crassostrea gigas* (Thunberg, 1793) and *C. virginica* (Gmelin, 1971). *Jour. of Shellf. Res*. 8 (1): 117-123.

Wistreich, G.A. 1997. Microbiology Laboratory: Fundamentals and Applications. Prentice Hall Inc. New Jersey.

Wong, T.M. 1990. Seed production of local bivalves via hatchery techniques. Proceedings from 1st. Malaysian Applied Science Symposium. Universiti Putra Malaysia, Serdang Malaysia. February 1990.niversiti Malaya, Kuala Lumpur. 18 November 1989.

Wong, T.M., Tan, S.H. and Cheong, T.K. 1989. Induced spawning, larval development and early spat growth of the tropical oyster *Crassostrea belcheri* in the laboratory. 12th Marine Science Seminar.

Wright, R.T., Coffin, R.B., Ersing, C.P. and Pearson, D. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. *Limnol. Oceanogr.* 27: 91-98.

Yamamoto, H., Tachibana, A., Kawaii, S., Matsumura, K. and Fusetani, N. 1996. Serotonin involvement in larval settlement of the barnacle, *Balanus amphitrite*. *The Jour. of Exp. Zool.* 275: 339-345.

Yang, J-L, Li, X., Liang, X., Bao, W-Y., Shen, H-D. and Li, J-L. 2014. Effects of natural biofilms on settlement of plantigrades of the mussel *Mytilus coruscus*. *Aquaculture*. 424-425: 228-233.

Yang, J-L., Li, S-H., Li, Y-F., Liu, Z-H., Liang, X., Bao, W-Y. and Li, J-L. 2013. Effects of neuroactive compounds, ions and organic solvents on larval metamorphosis of the mussel *Mytilus coruscus*. 2013. *Aquaculture*. 396-399: 106-112.

Young, T., Alfaro, A.C. and Robertson, J. 2011. Effect of neuroactive componds on the settlement of mussel (*Perna canaliculus*) larvae. *Aquaculture*. 319: 277-283.

Yu, X., He, W., Li, H., Yan, Y. and Lin, C. 2010. Larval settlement and metamorphosis of the pearl oyster *Pinctada fucata* in response to biofilms. *Aquaculture*. 306: 334-337.

Yu, X., He, W., Gu, H-J., He, M. and Yan, Y. 2008. The effect of chemical cues on settlement of pearl oyster *Pinctada fucata martensii* (Dunker) larvae. *Aquaculture*. 277: 83-91.

Zardus, J.D., Nedved, B.T., Huang, Y. Tran, C. and Hadfield, M.G. 2008. Microbial biofilms facilitate adhesion in biofouling invertebrates. *Biol. Bull*. 214: 91-98.

Zhao, B., Zhang, S., Qian, P-Y. 2003. Larval settlement of the silver- or goldlip pearl oyster *Pinctada maxima* (Jameson) in response to natural biofilms and chemical cues. *Aquaculture*. 220: 883-901.