UNIVERSITI PUTRA MALAYSIA

EMBRYONIC, LARVAL DEVELOPMENT, GROWTH PERFORMANCE AND SALINITY TOLERANCE OF Crassostrea belcheri Sowerby AND Crassostrea iredalei Faustino HYBRIDS

WAN NUR FATIN SYAFIQAH BT WAN NAWANG

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

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Chair : Annie Christianus, PhD
Faculty : Agriculture

Crassostrea belcheri and *C. iredalei* are two species of tropical oysters found in Malaysian waters. Between these two oysters, *C. iredalei* is preferred by local consumers due to its tasty and creamy flesh. However, the disadvantage is being small in size. Thus, this study was carried out to hybridize these two oysters, to see whether the resulted hybrids are better in terms of flesh quality, size, growth and survival. Inter-specific hybrids between *C. belcheri* and *C. iredalei* were produced in the hatchery of Fisheries Research Institute (FRI), Pulau Sayak, Kedah to assess its potential as an aquaculture species in Malaysia. The specific objectives of these studies were to 1) observe and describe the embryonic and larval development, 2) to evaluate the growth performance, 3) to determine the salinity tolerance of spats, and 4) to determine the protein and fatty acids content of 8 month-old oysters of parental and inter-specific crosses of *C. belcheri* and *C. iredalei*. Broodstock of *C. belcheri* and *C. iredalei* were collected from Batu Lintang, Kedah. Parental and inter-specific crosses were produced through mechanical stripping. Parental and inter-specific crosses between *C. belcheri* (CB) and *C. iredalei* (CI) resulted in crosses of CB♀XCB♂, CI♀XCI♂, CB♀XCI♂ and CI♀XCB♂. Successful fertilization in both inter-specifics crosses confirmed that *C. belcheri* eggs can be fertilized by *C. iredalei* sperm and vice versa. The fertilization percentage of inter-specific crosses of CB♀XCI♂ (64.77±1.38%) and CI♀XCB♂ (30.23±2.51%) were significantly lower ($p<0.05$) compared to parental *C. belcheri* (80.69±1.53%) and *C. iredalei* (80.05±2.56%). Larviculture were carried out in 300 L tank in triplicates for each crosses. Percentage of survival of all crosses from each larval stages were measured at the end of the study period. However, there were no significant differences in survival (%) of each larval stage in all crosses. Similarly, no significant different ($p>0.05$) of survival was observed from fertilized eggs to D-stage in inter-specific crosses. Percentage of survival during transition period from D-stage to pediveliger stage in inter-specific crosses of CB♀XCI♂ was highest ($p<0.05$) 25.15±3.67%, followed by parental *C. belcheri* 16.8±0.38% and *C. iredalei* 21.02±1.73%, respectively. Pediveliger stage was observed the earliest at 11 days post-fertilization in CI♀XCB♂ while in the other

crosses at 13 days post-fertilization. Earliest settlements were observed in CI♀XCB♂ crosses at 12 days post-fertilization, followed by other crosses at 14 days post-fertilization. The percentage of fertilization of eggs from CI♀XCB♂ was lowest as compared to other crosses, however faster settlement and metamorphosis were observed. This finding shows that the hybrid progeny has a shorter planktonic stage as compared to progeny from parental crosses. Specific growth rate (SGR) was measured at 3 stages; planktonic (1-13 days), early settlement (13-30 days) and spat (30-100 days) stage. Progeny from CI♀XCB♂ shows highest ($p<0.05$) SGR and larger in size possibly due to early settlement. At settlement stage, spats from CB♀XCB♂ parental cross showed highest ($p<0.05$) SGR. Spats of 100 day-old were comparatively larger in inter-specific crosses as compared to spats from CI♀XCI♂. Thus, the progeny from the inter-specific crosses produce comparatively better growth and survival from the planktonic to spat stage. As for salinity tolerance, 3 experiments were conducted. Experiment 1 was conducted to determine the growth and salinity tolerance in 10, 20 and 30 ppt in 40 day-old spats. Experiment 2 was conducted to determine the percentage of spats survival exposed to short term salinity shock in 5, 10, 15, 20, 25, and 30 ppt. In Experiment 3, spats were exposed to three different salinity decrements, gradual, intermediate and rapid. Percentage of survival was determined at the end of experimental period. Spats were able to tolerate the drastic decrement in salinity evidence with 100% survival. Crude protein and fatty acids were measured in 8 month-old oysters. Crude protein was highest ($p<0.05$) in spats of CB♀CI♂ (56.09±0.64) followed by CI♀CB♂ (57.0±0.56), CB♀CB♂ (53.48±0.42) and then finally CI♀CI♂ (53.94±1.88). This study indicated that spats from inter-specific crosses CB♀CI♂ and CI♀CB♂ higher protein content as compared to parental crosses. Higher polyunsaturated fatty acids were observed in inter-specific cross of CI♀XCB♂. These results indicated that the hybrids offers better survival, growth, protein levels and fatty acids composition.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

EMBRIO, PERKEMBANGAN LARVA, PRESTASI PERTUMBESARAN DAN TOLERANSI KEMASINAN UNTUK *Crassotrea belcheri* (Sowerby) *Crassostrea iredalei* (Faustino) DAN KACUKANNYA

Oleh

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Pengerusi : Annie Christianus, PhD
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Crassostrea belcheri dan *C. iredalei* adalah dua spesis tiram tropika yang terdapat di perairan Malaysia. Di antara kedua-dua tiram ini, *C. iredalei* lebih digemari oleh masyarakat tempatan kerana isinya yang berlemak dan sedap. Walaubagaimanapun, kekurangannya adalah dari segi saiznya yang kecil. Oleh itu, kajian dijalankan dalam menghasilkan hibrid dari kedua-dua spesis tiram ini untuk melihat sama ada hibrid yang terhasil adalah lebih baik dari segi kualiti isi, saiz, tumbesaran dan kemandirian. Hibrid inter-spesifik di antara *C. belcheri* dan *C. iredalei* dihasilkan di hatceri Institut Penyelidikan Perikanan (FRI), Pulau Sayak, Kedah untuk menilai potensinya sebagai satu spesis akuakultur di Malaysia. Objektif spesifik kajian ini adalah untuk 1) memerhati dan menerangkan perkembangan embrio dan larva, 2) menilai pertumbuhan, 3) menentukan toleransi saliniti untuk spat, dan 4) menentukan kandungan protein dan asid lemak tiram berumur 8 bulan dari kacukan induk dan inter spesifik *C. belcheri* dan *C. iredalei.* Induk *C. belcheri* dan *C. iredalei* diambil dari Batu Lintang, Kedah. Kacukan induk dan inter spesifik dihasilkan melalui pelurutan secara mekanikal. Kacukan induk dan inter spesifik di antara *C. belcheri* (CB) dan *C. iredalei* (CI) menghasilkan CB♀XCB♂, CI♀XCI♂, CB♀XCI♂ dan CI♀XCB♂. Kejayaan persenyawaan dari kedua-dua kacukan inter spesifik menunjukkan bahawa telur *C. belcheri* boleh disenyawakan oleh sperm *C. iredalei* dan sebaliknya. Peratus persenyawaan kacukan inter spesifik CB♀XCI♂ (64.77±1.38%) dan CI♀XCB♂ (30.23±2.51%) adalah ketara lebih rendah ($p<0.05$) berbanding dengan kacukan induk *C. belcheri* (80.69±1.53%) dan *C. iredalei* (80.05±2.56%). Kultur larvi dijalankan dalam tripikat di dalam tangki 300 L untuk setiap kacukan tersebut. Peratus kemandirian untuk semua kacukan dari setiap peringkat larvi ditentukan pada akhir tempoh kajian. Walaubagaimanapun, tiada perbezaan ketara pada peratus kemandirian untuk setiap peringkat larvi tersebut untuk semua kacukan. Perbezaan tidak ketara ($p<0.05$) juga diperhati pada peratus kemandirian dari telur yang tersenyawa hingga ke peringkat D dalam kacukan inter spesifik. Peratus kemandirian semasa tempoh peralihan dari peringkat D ke pediveliger dalam kacukan inter spesifik CB♀XCI♂ ketara paling

tinggi ($p<0.05$) 25.15±3.67%, diikuti dengan kacukan induk *C. belcheri* 16.8±0.38% dan *C. iredalei* 21.02±1.73%, masing-masingnya. Peringkat pediveliger diperhati paling awal pada 11 hari selepas persenyawaan dalam CI♀XCB♂, manakala 13 hari selepas persenyawaan untuk kacukan yang lain. Pendudukan yang paling awal adalah pada kacukan CI♀XCB♂ iaitu 12 hari selepas persenyawaan, diikuti dengan kacukan yang lain pada 14 hari selepas persenyawaan. Peratus persenyawaan telur dari CI♀XCB♂ adalah yang terendah berbanding dengan kacukan yang lain, walaubagaimanapun, ianya menunjukkan pendudukan dan metamorfosis yang paling cepat. Hasil kajian ini menunjukkan bahawa progeny hibrid mempunyai peringkat planktonic yang paling pendek berbanding dengan progeni kacukan induk. Kadar pertumbuhan spesifik (SGR) diukur pada 3 peringkat, iaitu planktonic (1-13 hari), pendudukan awal (13-30 hari) dan spat (30-100 hari). Progeni dari CI♀XCB♂ menunjukkan SGR tertinggi ($p<0.05$) dan dengan saiz yang lebih besar mungkin disebabkan oleh pendudukan yang lebih awal. Pada peringkat pendudukan, spat kacukan induk CB♀XCB♂ menunjukkan SGR yang paling tinggi ($p<0.05$). Spats berumur 100 hari adalah lebih besar dalam kacukan inter spesifik berbanding dengan spat CI♀XCI♂. Oleh itu, progeny dari kacukan inter spesifik menghasilkan tumbesaran dan kemandirian yang lebih baik dari peringkat planktonic ke spat. Sementara untuk toleransi saliniti, 3 eksperimen telah dijalankan. Eksperimen 1 dijalankan untuk menentukan tumbesaran dan toleransi saliniti untuk 10, 20 dan 30 ppt untuk spat berumur 40 hari. Eksperimen ke 2, dijalankan untuk menentukan peratus kemandirian spat yang didedahkan pada kejutan saliniti jangka pendek pada 5, 10, 15, 20, 25, dan 30 ppt. Dalam eksperimen ke 3, spat didedahjkan kepada 3 jenis penurunan saliniti, iaitu secara beransur-ansur, intermediat dan mendadak. Peratus kemandirian ditentukan pada akhir tempoh kajian. Spat boleh bertoleransi dengan penurunan saliniti yang mendadak ternyata dengan kadar kemandirian 100%. Protein kasar dan asid lemak diukur pada tiram yang berumur 8 bulan. Protein kasar adalah tertinggi ($p<0.05$) pada spat CB♀CI♂ (56.09±0.64) diikuti dengan CI♀CB♂ (57.0±0.56), CB♀CB♂ (53.48±0.42) dan akhir sekali CI♀CI♂ (53.94±1.88). Kajian ini menunjukkan bahawa spat dari kacukan induk iaitu CB♀CI♂ dan CI♀CB♂ mempunyai kandungan protein yang lebih tinggi berbanding dengan induk. Asid lemak politaktepu adalah tinggi pada didapati pada kacukan inter spesifik CI♀XCB♂. Keputusan menunjukkan bahawa kacukan inter-spesifik menunjukkan kadar hidup, tumbesaran, kandungan protein dan asid lemak politaktepu lebih baik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of degree of Master of Science. The members of the Supervisory Committee were as follows:

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DSP	Diarrhetic shellfish poisoning
%	Percentage
˚C	Celsius
µm	Micrometer
♀	Female
♂	Male
AA	Arachidonic acid
ALA	A-Linolenic acid
BOBP	Bay Of Bengal Programme
C14-C22	Carbon 14- carbon 22
CB	*Crassostrea belcheri*
CB♀XCB♂	*Crassostrea belcheri* female X *Crassostrea belcheri* male
CB♀XCI♂	*Crassostrea belcheri* female X *Crassostrea iredalei* male
CI	*Crassostrea iredalei*
CI♀XCB♂.	*Crassostrea iredalei* female X *Crassostrea belcheri* male
CI♀XCI♂	*Crassostrea iredalei* female X *Crassostrea iredalei* male
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
ELH	egg-laying hormone
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FRI	Fisheries Research Institute
g	Gram
GC	Gas Chromatography
GLA	Gamma-linolenic acid
H_2SO_4	Sulfuric acid
KOH	Potassium hydroxide
L	Liter
LA	Linoleic acid
ml	Millimeter
mm	Millimeter
MUFA	Monounsaturated fatty acids
PLSM	Plasmaglogen
PPT	Part per thousand
PSP	Paralytic shellfish poisoning
PUFA	Polyunsaturated fatty acids
SAT	Saturated fatty acids
SGR	Specific growth rate
SOP	Specific Operating Procedure
Sp.	Species
TSS	total suspended solid
WoRMS	World Register of Marine Science

CHAPTER 1

INTRODUCTION

1.1 General background

Most of marine mollusc exhibit a complex separated phase of life cycle which are planktonic phase and benthic phase (Thorson, 1950). According to Hedgecock (1995), the planktonic larval of oyster can be found near at open coastal water. During this prolonged stage, they quite sensitive to the environmental changes. The earliest planktonic is the most vulnerable and susceptible phase in mollusk life cycle before they metamorphosed into benthic juvenile (Bayne *et al.,* 1976). Their environmental preference is very limited (Claudi and Evans, 1993). In comparison, the juvenile (spat) and adult oyster able to withstand broad fluctuation of temperature, pH, dissolve oxygen, salinity, and suspended sediments (Buroker, 1983; Tan and Wong, 1996). Oyster varies in shape and this morphologically characteristic is very much affected by environmental fettle (Tack *et al.,* 1992). Their shape also depends on the attachment of post-metamorphosis process. Thus, identification based on phenotypic of a species irrelevant due to varied shape and form.

Oyster is from the phylum mollusc and class bivalvia. These invertebrates have shells which act as an armor for soft-body tissues. Their shells were held by a valve on the hinge that acts as ligament during opening and closing. Generally, benthic mollusk plays important roles in ecosystem as filter feeder. Oyster obtains food by filtering seawater and their growths totally depend on microalgae (Berg and Newell, 1986). *Crassostrea virginica* is an eastern oyster which able to filter 60 gallon of water daily (Chrismas and Jordan, 1987).

When oyster grow to form population, they are able to alter the biotic and abiotic surrounding (Jones *et al*., 1994). Due to their capability as good filter feeders, they directly filtered phytoplankton, they also known as bio-indicator for pollution in an ecosystem by measuring their heavy metals content (Lim *et al.,* 1995). However these heavy metals can adversely affect their larvae evidence with abnormalities in feeding, swimming behavior, development and metamorphosis Calabrese *et al.,* 1973). Even though oyster is a hardy species, they can be susceptible by prolonged duration of low salinity, temperature fluctuations and low dissolve oxygen (Pernet *et al*., 2010).

In an ecosystem, the chemical and physical characteristics are affected by epifaunal species (Pearson and Rosenberg, 1978). Most of the bivalve beds can be found at estuaries areas that offer good biological filtration for biodiversity (Palmer, 1980; Pouvreau *et al*., 1999). Most bivalve is able to survive with high tolerance of pollution and low dissolve oxygen due to their significant capability as biological filter for water (Davis, 1975; Pouvreau *et al*., 1999). Oyster vigorously filtering the water from open water column and effectively remove suspended particles as for food sources and energy consumption for growth (Kuenzler, 1961; Paul *et al*., 1978; Officer *et al.*, 1982). Their capabilities to eliminate and coagulate the unwanted particle significantly enhance them to survive well at these polluted areas (Meyer *et al*., 1997).

In estuaries, oyster has high capabilities to exhibit good growth through coagulation mechanism by coagulating the unwanted particles to waste product (Officer *et al.*, 1982). Thus, their existence is significant in the ecosystem due to their vital roles to filter water particles matter for water improvement such as water clarity for better light penetration (Meyer *et al*., 1997). Additionally, they also act as stabilizer for sedimentation (Wells, 1961; Peterson *et al*., 2003). Their filtration rate is closely correlated with abiotic factor such as temperature and salinity. Bivalves have high tolerance to temperature and salinity through the manipulation of their metabolism (Thorsen, 1936). Consequently, these factors affect their metabolic rate and growth (Bullock, 1955).

Naturally, oyster is a biological indicator for pollution in an ecosystem. It has the capabilities to filter the phytoplankton in water column and depute pollutions in flesh and shells especially heavy metals. (Lim *et al.*, 1995). Capabilities of bivalve in concentrating the metals in high magnitude, poses an adverse effect to human when consumed (Lim *et al*., 1998). Heavy metals have their own biological importance as trace element. Living organism need a small amount of heavy metals for good health (Choupagar, 2011). Nevertheless, high level of heavy metals is poisonous and can lead to health problems. It persistent presence in environment since it cannot be destroyed or degraded (Duruibe *et al*., 2007).

Higher number of bivalve consumption can lead to shellfish poisoning. This is caused by dinoflagellate algae from the genus *Alexandrium* known that able to synthesize neurotoxin resulting in paralytic shellfish poisoning (PSP) toxin (Teegarden and Cembella, 1996). *Dinophysis* spp. also can give adverse effects to human, causing diarrhea through the production of diarrhetic shellfish poisoning (DSP) (Cordier *et al.*, 2000). Shellfish poisoning can occurs since shellfish are capable to aggregate the domoic acid a neurotoxin produced through their filter feeding on a certain strain of phytoplankton. This neurotoxin is harmful towards human (Jeffery *et al*., 2004).

In nature, oyster juveniles can be found attached to substrates along estuaries such as aerial mangrove roots, rocks, beam of concrete, plastic drum or other permanent structure (Devakie and Ali, 2002). The settlement of bivalve is triggered by internal and external factor such as chemical and type of substrate, water quality, nutritional consumption and genetic heredity (Hadfield, 1984). According to Ng (1979), there are four genera of oyster found in Malaysian coastal water which include *Crassostrea* (*Crassostrea belcheri*, *Crassostrea iredalei*), *Saccostrea* (*Saccostrea* spp and *Saccostrea echinata*), *Ostrea* (*Ostrea folium*), and *Hyotissa* (*Hyotissa hyotis*). However, FAO (2013) reported five species found in Malaysia which include *Crassostrea belcheri*, *Crassostrea rivularis*, *Saccostrea cucullata*, and *Ostrea folium*. Identification of these oysters based on phenotypically is extremely difficult. Thus, researcher suggested that identification of oyster under the genus *Crassostrea* using molecular method (Klinbunga *et al.,* 2003; Lam and Morton, 2003). Nevertheless, the diversity of oyster species in Malaysia is rather low when compared to other country (Kam, 2009).

Oyster from genus *Crassostrea* are euryhaline during planktonic and benthic phase (Quayle and Newkirk, 1989). During annual monsoonal season, the salinity at estuaries can fluctuate to as lower as 3-5 ppt for 7-8 days, while it can go extremely high during dry season (Tan and Wong, 1996). These species are able to tolerate wide temperature change between 25-32˚C in low diurnal change in Malaysia (Angell, 1989). However, this temperature can lethal for *Crassostrea* species when it reaches 41-44˚C (Davenport and Wong, 1992). Comparable to other species of oyster, the changes of salinity can initiate natural spawning (Chin and Lim, 1975). Nevertheless, some of these species can spawn continuously throughout the year (Giese and Pearse, 1974). Hence, spawning season for these species in tropical region is difficult to predict. Thus, the consistently seeds supply from wild is not reliable to fulfill demand by farmers (Devakie and Ali, 2002). Therefore, it is crucial to ensure continuous supply of oyster's seedling through proper and broodstock management and conservation programs (Hedgecock and Okazaki, 1984).

Biological activity of oyster is commonly related to the environment and ecosystem (Purchon, 1968). According to Galtsoff, (1964), activities such as embryonic and larvae development, growth, feeding, gonadal maturation, spawning, settlement and metamorphose of oyster depend on physicals and chemicals factors of the environment. These environmental factors are influenced by biotic and abiotic characters of the ecosystem such as salinity, temperature, pH, total suspended solid, dissolve oxygen, food availability (Van *et al.*, 2004). Temperature is known as significant factor controlling for gametogenesis (Mann, 1979; Chaves-Villalba *et al.*, 2002; and Fabioux *et al*., 2005). Photoperiod and temperature are two crucial key factors for oyster to trigger reproductive development (Fabioux *et al*., 2005; Pouvreau *et al*., 2006). Gametogenesis in *Crassostrea* sp. is controlled by both endogenous physiological factors and exogenous signals from the environment (Ren and Ross, 2001; Fabioux et al., 2005; Pouvreau *et al*., 2006; Dridi *et al.*, 2007). The chemical properties of water parameter in an environment act as cues for larvae to colonize during settlement (Johnson and Strathmann, 1989; Jensen and Morse, 1990).

Crassostrea belcheri can be found at the intertidal zone of an estuary in Malaysian coastal water where they occasionally attach to aerial roots of mangrove tree (Tan and Wong, 1996). *Crassostrea belcheri* is therefore often called as a tropical mangrove oyster with wide range of salinity and temperature preferences (Tan and Wong, 1996). In Kedah, *C. belcheri* was found attached to plastic drum used for fish cage (aquaculture site) and on jetty structure in Batu Lintang, Kedah. *Crassostrea iredalei* is preferable by local communities due to it high index contain and more tasty flesh, and milky white in color.

1.2 Current status of oyster in Malaysia

Oysters belong to the genus *Crassostrea* are being commercialized by the economic sector. Even with oyster from the genus *Ostrea* and *Saccostrea* found in abundance the Malaysian coastal water, the demand for oyster from genus the *Crassostrea* is higher. This is basically due to their capability to growth faster, high condition index, and easily harvested from nature. According to Department of Fisheries Malaysia (2013), the retail value of bivalve production in Malaysia increased from 2012 to 2013 from 695.20 to

3,453 tons annually. Nevertheless, oysters only contributed about 1.4% of the aquaculture production in Malaysia. Researches were carried out by The Fisheries Research Institute (FRI), Pulau Sayak, Kedah to improve the quality and production of oyster. Presently, the general public in Malaysia is still unaware of the health benefits of through the consumption of oyster.

In Malaysia, oyster culture is not well established. Oyster production is still through conventional practice. Efforts by the Department of Fisheries Malaysia to disseminate propagation of oyster under BOBP funding have significantly increased the production of oyster. Traditionally, spats were collected using coconut shell, bamboo pole, oyster shell, asbestos and old tires (Devakie, 1993). Oyster culture practices in Malaysia are with using, bottom, rack, and raft system. *Crassostrea belcheri* cultures are located at Muar River, Johore, Sandakan, Tawau and Labuan, Sabah (FAO, 2013). In Sarawak, *C. rivularis* and *Saccocstrea cucullata* are culture using raft and rack technique. *Ostea folium* a small sized oyster is culture at Pulau Langkawi, Kedah using raft and rack technique (FAO, 2013).

Presently, spats produced by the Fisheries Research Institutes (FRI) in Pulau Sayak, Kedah were distributed to farmers. Several researches reported the induction of metamorphosis timing using different type of substrate and substrate conditioning for a good attachment of juvenile oyster (Nikilic *et al.*, 1976; Alagarswami *et al.*, 1983; Devakie and Ali, 2002; Buitrago and Alvarado, 2005; Ehteshami *et al.,* 2011; Tanyaros, 2011). Successful of settlement and metamorphosis of mollusk significantly depend on substrate (Pawlik, 1992). Thus, suitable substrate is important to improve survival and growth of juvenile. In Malaysia, the spawning season is quite unpredictable. Therefore, the collector placed randomly in the open water column of the estuaries.

Attempts to increase the production and improving the survival of oyster in Malaysia were carried out through the manipulation of salinity and temperature level (Davenport and Wong, 1992; Tan and Wong, 1996; Devakie and Ali, 2000b), feeding regime (Devakie and Ali, 2000a), metamorphosis timing using chemicals (Teh *et al.*, 2012) and used of physical and chemical substrate to enhance the larval settlement and reduce planktonic period (Hadfield, 1984; Holiday, 1996; Taylor *et al.*, 1997; Devakie and Ali, 2002). In order to overcome these constraints, the seed should be produced in hatchery for aquaculture purposes (Tan and Wong, 1996).

1.3 Problems statement

The main problem in the supply of oyster seedlings in Malaysia is due to the ambiguous status of the spawning season. In Malaysia, *Crassostrea* sp is able to spawn throughout the year. Main factor initiating this spawning is salinity and temperature (Angell, 1986). Heavy spat fall is estimated during the decrement of salinity fluctuation. Naturally, the spats are collected during monsoon season. Rafts are installed before the onset off spawning season. It’s important to identify the spawning season for oyster to reduce the attachment of bio-fouling organism on the collectors (Dangwatanakul, 1992). Oyster spats are left for 4-6 months to grow to reduce mortality during harvesting process.

However, due to the different spawning season resulted in the difficulty in estimating the collection of wild spats. Thus, the inconsistent of seed supply from the wild is insufficient to support and fulfill the demand (Devakie and Ali, 2002). Hatchery production is therefore more reliable. Seed supply is vital for shellfish aquaculture (Hedgecock and Okazaki, 1984). Therefore, the seeds of oyster are produced from hatchery to support the demand from consumers. Currently in Malaysia, the seeds were harvested from nature and the clutch was installed for settlements. However, due to ambiguous status of spawning season, water quality and salinity tolerance of oyster at the different stage of life. The spats are inadequate from wild and yield small number to fulfill the production of oyster. Thus, the seeds were imported from nearby country to sustain the demand for oyster culture in Malaysia (Devakie and Ali, 2002).

Thus, this scenario encourage researcher to find other alternative to overcome the limited spats supply. Researches draw closer with other option for example triploidy, chemical and neuron active compound application for settlement (Young *et al*., 2011; Sánchez-Lazo and Marínez-Pita, 2012: Wassnig and Southgate, 2012), bacteria and biofilm (Yu *et al*., 2010; Abee *et al.,* 2011), biochemical composition at the different season (Martino and Cruz, 2004; Dridi *et al.*, 2007), diet preference at the different stage of life (Caers *et al.,* 2003; Villa *et al*., 2006), substrate (Devakie and Ali, 2002), pheromones application (Bishop *et al*., 2006) and so on. Conversely, various studies related to the application of chemical contribute to adverse affect for spats especially for growth and survival for the early stage of larvae.

The comparison between these two species of *Crassostrea belcheri and C. iredalei* were size and texture of the flesh (Devakie and Ali, 2002). The *Crassostrea iredalei* was highly recommended due to their high condition index and favorable taste for consumers. However, the size and slow growth is the main problems since they are smaller compared to *C. belcheri*. *Crassostrea belcheri* offers fastest growth and larger size with the chewy texture of flesh. However, the lower condition index and unfavorable texture results lower consumption on this species. Thus, the inter-specific between these two species is predicted to produce better quality without any chemical introduce to produce new breed of good quality of oyster.

Water quality parameter plays vital roles in shellfish aquaculture. The United State Shellfish Sanitation Standard was introduced for shellfish aquaculture practice to reduce from adverse affects towards consumers. The import or export of any shellfish will followed the regulations of Shellfish Sanitation Standard and conditionally approved for further activities (Dinamani, 1991). Thus, the management which include harvesting, amount of the rains fall that highly caused water runoff from any area, equipment, transportation and handling also need to fulfill the criteria for export (Dinamani, 1991). This sanitation regulation can helps to reduce the shellfish poisoning to consumers.

The main risk for oyster is due to predation (Werner and Mittlebach, 1972). The mudworm, *Polydora websteri* is the main predator attacking these oysters (Dinamani, 1991). They are capable to drill the shell, and leave a tunnel trail on the shell which may also affect the meat quality of oyster. Thus, these oysters are not fit for market. The pea crab, *Pinnotheres* sp and copepod, *Psedomyicola* sp. are two species found in the mantle cavity of the oyster. This copepod will invade the gut and caused disease in oyster. In addition, dinoflagellate algae also can contribute to constraint of bivalve. These dinoflagellate microalgae of the genus *Alexandrium* is able to cause paralytic shellfish poisoning (PSP) towards consumer (Teegarden and Cembella, 1996). Thus, when it comes in contact with human, it can cause diarrhea. Similar symptom also can be due to *Dinophysis* spp. Generally, the phytoplankton is food sources for oyster. However, the capability of certain phytoplankton to trigger the neurotoxin is harmful to consumer (Jeffery *et al.*, 2004).

1.4 Significant of study

The major difficulty in oyster farming is wild spats supply for grow out. The significant of this study, inter-specific crosses progeny offers good quality of new hybrid species exhibit better growth, survival, and reduce timing settlement. Thus, the insufficient spat can be overcome and aid spat production in hatchery for farming purpose. The application of chemical and physical treatment for enhance the cue in settlement can be solve by promoting the new inter-specific crosses offers shortening the settlement process. Therefore, the application of chemical or neuroactive compound economically can be reducing and the uses of chemical cause adverse effect to larvae and spats.

1.5 General objective

The objectives for these studies were to:-

1. Observe describe the embryonic development of parental and inter-specific crosses of *C. belcheri* and *C. iredalei*

2. To evaluate the growth performance of the parental and inter-specific crosses of *C. belcheri* and *C. iredalei*

3. To determine the salinity tolerance and its decrement of spats of parental and inter-specific crosses

4. To determine the protein and fatty acids content of 8 month-old spats from parental and inter-specific crosses *in* of *C. belcheri* and *C. iredalei*

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PUBLICATIONS

Wan Nawang W.N.F.S., Christianus A., Ehteshami F., Taha M.S.M., and Jamari Z., (2019). Development of *Crassostrea belcheri* (Sowerby, 1871), *Crassostrea iredalei* (Faustino, 1932) and inter-specific cross spats at different salinity. *Journal of Survey in Fisheries Sciences*, 6(1), 77-87.

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