Production of tropical oyster seed in hatchery

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Abstract

Oysters have been collected from wild since long time ago for human consumption. Recent development in aquaculture has allows the production of its seed in hatchery. Factors favoring the oyster production are stocking density, water quality and the availability of live food. These factors can increase the growth and survival of the oyster. Fundamental knowledge on life cycle and biology of oyster is important as it can become the basis for successful development of oyster culture. Due to the limited seed supply, hatchery produce seedlings are important to support the development of oyster industry.

Keywords: Life cycle, Microalgae, Oyster culture, Propagation, Stocking density

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Introduction

Little information is available on the biology and culture of native oyster in Malaysia. In early 1960's by the Department of Fisheries Malaysia favored by a Colombo Plan Expert has conducted some studies on oyster. During mid-seventies, experimental culture of *Crassostrea belcheri* was carried out in Sabah (Chin and Lim, 1978). Then, followed by cultured of flat oyster, *Ostrea folium* in Pulau Langkawi (Ng, 1979). The culture system uses polyethylene net as substrate for oyster attachment. In 1988, Department of Fisheries Malaysia with the cooperation of Bay of Bengal Programme (BOBP) started a more aggressive approach for the production of oyster. Under this program, suitable spatfall and areas for culture were studied intensively. Thus, the establishment of oyster production in areas at Sg. Muar (Ng, 1979), Langkawi, Penang, Pangkor and Melaka (Angell, 1988). However, the production of oyster in Malaysia is still very dependent on seedling collected from the natural sources (Ng, 1991).

Basically, four genera of oysters considered native to Malaysia are *Crassostrea iredalei* (Fig. 1a), *C. belcheri* (Fig. 1b), *Ostrea folium*, *Saccostrea cucullata* and *Hyotissa hyotis* (Lam and Morton, 2009). Among these oysters, *C. iredalei* has the highest commercial value due to its taste and appearance (Mohd Yatim, 1993). Oysters have high demand as food in restaurant and steamboat stall. Two common species cultured by fishermen on commercial basis are *C. iredalei* and *C. belcheri*. Their spats are usually collected from the wild. In recent years, the limited supply of seeds from the coastal waters of Malaysia causes the decline in the production of these two oysters. In order to increase this production, the Fisheries Research Institute (FRI) and Universiti Sains Malaysia (USM) have conducted a collaborative research for the artificial propagation of *C .iredalei* and *C. belcheri* in hatchery. The first successful spawning and larvae rearing of *C. iredalei* was reported in 1990 (Wong, 1990).

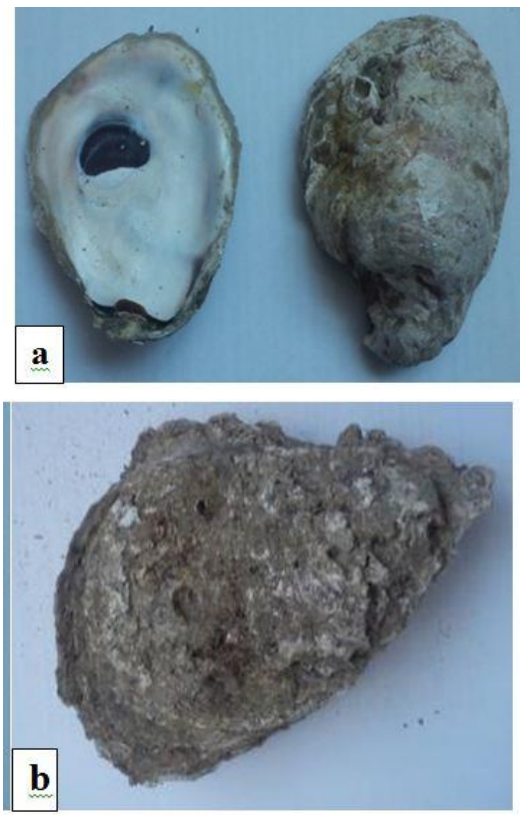

Figure 1: Oysters species in Malaysia, *Crassostrea iredalei* (a) and *Crassostrea belcheri* (b).

Oyster is the most valuable edible mollusks belong to the family Ostreidae.

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The irregular shell and varies shape depending on its attachment to substrate. The upper valve is small and flat while the lower valve bigger, convex and slightly excavated. The inside surface of the shell is smooth and chalky white. Upper and lower valves are connected to each other at the toothless hinge (Rosell, 1990). The colors of scar depend on species. Internal anatomy showed the position of abductor muscle (Fig. 2).

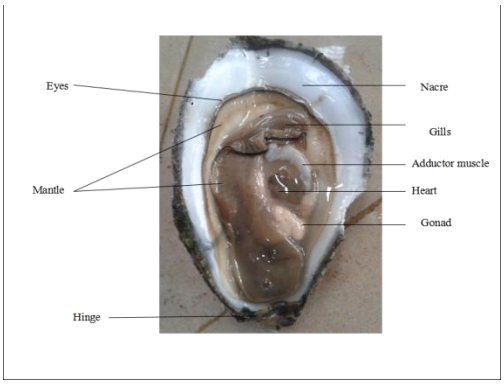

Figure 2: Internal anatomy of tropical oyster, *Crassostrea iredalei*.

According to Visootiviseth (1998), *Crassostrea iredalei* has purple-black adductor muscle scar, whereas white in *C. belcheri* (Fig. 3).

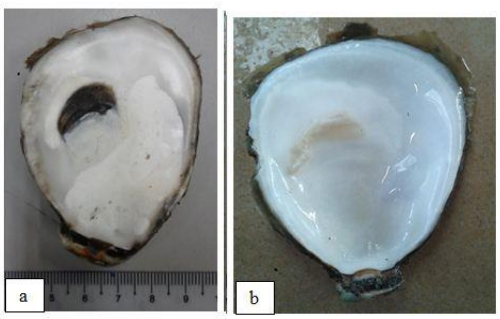

Figure 3: Color of scar in *Crassostrea iredalei* (a) and *Crassostrea belcheri* (b).

Similarities in the morphological structures, such as in *C. madrasensis* and *C. iredalei* (Suzana *et al.*, 2011), often resulted in the misidentification of oyster species (Lam and Morton, 2003; Yu *et al.*, 2003; Xia *et al.*, 2008). Thus, the used of molecular technique has been applied for phylogenetic and taxonomic study of oyster (Xia *et al*., 2008; Wang *et al.*, 2010; Suzana *et al.*, 2011).

Maturation and spawning

Most of the tropical oysters in Malaysia are protandric hermaphrodites. This allows it to change from male to female upon reaching adult stage and vice versa (Blanco *et al.*, 1951). Oyster can reach sexual maturity at the size of 50 to 80 mm (Rosell, 1990). Creamy white color indicates of ripe gonad, therefore is ready to spawn. Fertilization occurs externally with sperms being released continuously as dense white streams, and eggs released aided by contraction of adductor muscles. A single female can release millions of eggs. The tropical climate of Malaysia allows oysters to spawn throughout the year. The peak spawning seasons is between April to June and October to December (Devakie *et al.*, 1993). The temperature and salinity changes during these seasons trigger the oysters to spawn actively.

Artificial propagation can be carried out in hatchery by taking ripe broodstock from grow-out site or from wild. Broodstock shell are cleaned from barnacle, worm and other organism. Immersion with 10-20 ppm calcium hypochloride for 20 to 30 minutes will

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further eliminates protozoa and other organism on the shells. According to Ng (1993), selection of spawners is based on gonad maturity (Fig. 4).

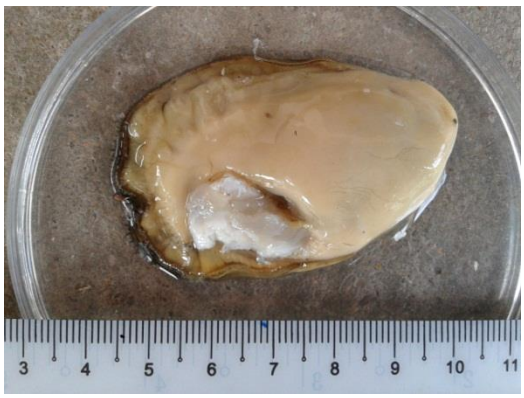

Figure 4: Matured oyster gonad.

Natural spawning can be conducted by placing oysters in spawning tank supplied with ultra-violated (UV) treated running seawater. The release of sperms triggers the female to ovulate. As for selective breeding, the broodstock can be placed in separate tank, then sperms and eggs are collected and subsequently fertilized. For both techniques, the fertilized eggs are collected then placed in incubation tank for 24 hours (Ng, 1993). Artificial spawning can be carried out by treating the broodstocks with chemical such as serotonin. This chemical triggers the broodstock to release the sperms and eggs for fertilization (Gibbon and Castagna, 1984; Velasco *et al.*, 2007).

Embryonic and early development

The pelagic phase of oyster larvae can last for approximately 20 to 22 days. The haploid sperm and egg fused when fertilization occurs to form a zygote. When the polar body appeared, it indicates successful fertilization. Thirty minute after fertilization, the eggs start to divide and developed into two-celled stage. Division of cells increase the weight of eggs. Eggs developed further into multi-celled blastula, gastrula stage and moving trochopores within 24 hours. Trochopores are oval in shape with size of 60 to 80 µm. These trochopores swim with the aid of cilia and long flagellum. Before it become fully-shelled D larvae in 24 hours, the velum will develop as a feeding organ. Whilst the larvae move, the velum will feed on phytoplankton in the water. Umbone will develop after thirteen days of fertilization. At umbo stage, the umbones are more prominent. Eye-larvae stage commences when a small dark circular dot appear at the center of each valve. At pediveliger stage, the foot and gills are developed. Larvae will then settle down as soon as they found a suitable substrate. They secrete the cement from the byssal gland at the foot for attachment. This cement hardens rapidly. Upon attachment to substrate, the foot disintegrates, then the oyster remains permanently at the selected substrate.

Larvae rearing and spat collection

Oysters are an epifaunal species with pelagic larval stage and sessile adult. Life stages include trocophore, umbo, D-shape, eye-stage, pediveliger, plantigrade, spat, juvenile and adult (Fig. 5). Generally, the growth and survival of oyster depend on temperature. According to Devakie and

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Ali (2000), *C. iredalei* can tolerate wide range of temperature from 24 to 36ºC. *Crassostrea iredalei* larvae set at temperature range of 10 to 20ºC and salinity of 30.2 to 40.1% (Devakie and Ali, 2000). Southgate and Lee (1998) reported that *Saccostrea echinata* can survive well when reared at 29ºC.

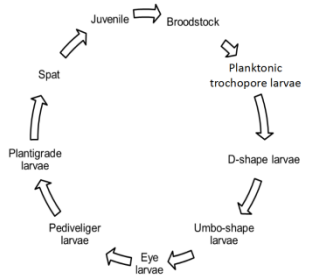

Figure 5: Life cycle of tropical oyster.

Stocking density can affect the survival of larvae. However, high density culture will increase competition for space, which may results in the increase larvae mortality. D-stage larvae with size of 50 to 60µm can be stocked at 5 larvae per mL. Stocking can be reduced further as the larvae grow. Recommended stocking for the larvae of different species of oyster is as shown in Table 1.

Microalgae culture

Microalgae have been used as food in bivalve hatcheries since 1940s (Bruce *et al.*, 1940). The development of each bivalve stage is fully dependent on diets of cultured microalgae such as *Chaetoceros calcitrans, Pavlova lutheri, Isochrysis* sp. and *Thalassiosira pseudonana* (Coutteau and Sorgeloos, 1992). These microalgae are used as food for larvae, juvenile and broodstock. In traditional culture, juveniles of 500 µm to 2-5 mm are transferred into tanks supplied with seawater from estuary. Thus, the juveniles are able to get food from the natural seston in water (Rodhouse *et al.*, 1981). This method incur low cost, but with high risk. Microalgae can be cultured using two techniques, traditionally with batch mode in carboys or modified technique using semi-continuous mode in alveolar photo-bioreactor (Ponis *et al.*, 2003). Instead of monospecific diet, various studies showed that multispecific diet is better for bivalve. This is because different species of microalgae has different nutritional profile. Thus, mix diet can complement each other to sufficiently support the growth of larvae. In mollusc hatchery, mass culture of larvae is hindered due to the limited supply of natural phytoplankton. High concentration of microalgae is needed in hatchery to support the optimum growth and survival of molluscs. Common species of microalgae use as food for oyster are as shown in Table 2.

Selection of microalgae as food for mariculture is based on cell size, shape, digestibility, and biochemical composition (Doroudi *et al.*, 2003; Martinez- Fernandez *et al.*, 2004). According to Chrétiennot-Dinet *et al.* (1991), the selected microalgae must have high nutritional value and can be mass cultured without much difficulty. Lipids are essential component in the food for all aquatic larvae, including bivalves.

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Table 1: Stocking density recommended for the culture of oyster larvae

Species	Stocking density (larvae/mL)	Size of oyster at initial stocking (µm)	Source
Pinctada margaritifera	1,2 or 5	81.5 ± 2.5	Doroudi and Southgate, 2000
Crassostrea iredalei	1	>250	Devakie and Ali, 2000
Pinctada margaritifera	2	81.5 ± 2.5	Doroudi *et al*., 1999
Pinctada margaritifera	4.1	81.3 ± 0.06	
Pinctada margaritifera	2	122.9 ± 0.09	
Saccostrea echinata	5	>210	Southgate and Lee, 1998

Table 2: Types of microalgae as food for oyster

Class of microalgae	Species of microalgae	Species of culture	Source
Prymnesiophyceae	*Pavlova salina* *Pavlova* sp. *Isochrysis* sp. clone TISO	*Pinctada margaritifera*	Martìnez-Fernández and Southgate, 2007
Bacillariophyseae	*Chaetoceros muelleri* *Chaetoceros* sp. *Skeletonema* sp.		
Prasinophyceae	*Micromonas pusilla*		
Prymnesiophyceae	*Isochrysis aff. galbana TISO* *Pavlova* sp. *Pavlova pinguis*	*Crassostrea gigas*	McCausland *et al.*, 1999
Bacillariophyceae	*C. calcitran* *S. costatum*		
Chlorophyceae	*Dunaliella tertiolecta*		
Cryptophyceae	*Rhodomonas salina*		
Prymnesiophyceae	*Isochrysis aff. galbana TISO* *P. salina*	*Pinctada margaritifera*	Doroudi *et al*., 1999
Prymnesiophycea	*Isochrysis galbana*	*Crassostrea iredalei*	Devakie and Ali, 2000
Bacillariophyceae	*Chaetoceros calcitran*		
Prymnesiophycea	*Isochrysis aff. galbana (TISO)* *Pavlova lutheri*	*Crassostrea gigas*	Brown and Robert, 2002
Bacillariophyceae	*C. Calcitran* *C. Calcitran forma pumilum* *C. muelleri* *Chaetoceros* sp. *Skeletonema costatum*		
Prymnesiophyceae	*Pavlova lutheri* *Isochrysis affinis galbana*	*Crassostrea gigas*	Rico-Villa *et al*., 2006
Bacillariophyceae	*C. calcitran forma pumilum*		
Prymnesiophyceae	*Isochrysis aff. galbana (TISO)*	*Crassostrea gigas* *Ruditapes philippinarium*	Delaporte *et al*., 2003
Bacillariophyceae	*C. calcitran*		
Prasinophyceae	*Tetraselmis suecica*		
Prymnesiophyceae	*Isochrysis aff. galbana (TISO)*	*Crassostrea gigas*	Rico-Villa *et al*., 2009
Bacillariophyceae	*C. gracilis* *S. marinoi*		
Bacillariophyceae	*C. calcitran* *C.muelleri*	*Pinctada margaritifera*	Ehteshami *et al*., 2011
Prymnesiophyceae	*Isochrysis* sp.		

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They are source of energy and form part of the biological membrane (Arts *et al.*, 2009). However, during digestion, lipids are hydrolysed into primary component called fatty acid. Thus, the nutritional value of microalgae is very much related to the specific composition of fatty acid than lipid (Webb and Chu, 1982; Delaunay *et al.*, 1993). The profile of fatty acids differ between microalgae. Certain microalgae are lack of essential fatty acid (Brown *et al.*, 1989; Volkman *et al.*, 1989). For instance, docosahexanoic acid (22:6(n-3), DHA) content in *I. galbana* is high, while low in eicosapentanoic acid (20:5(n-3), EPA). Conversely, *C. gracilis* is rich in EPA but deficient in DHA (Brown *et al.*, 1997; Ehteshami *et al.*, 2011).

Most microalgae used in bivalve hatcheries contained high nutritional value, especially in polyunsaturated fatty acid (PUFA) of the n-3 series EPA (eicosapentaenoic acid 20: 5n-3) and DHA (docosahexanoic acid 22:6n-3) respectively (Brown *et al.*, 1997). These PUFA are essential for marine animals (Kanazawa *et al.*, 1979). In bivalve hatcheries, controlled mass culture of microalgae required manpower and economic investment. In fact, microalgae production cost varies from 15 to 85 % of the overall hatchery management costs depending on scale of production and cultivation methods (Urban and Langdon, 1984; Coutteau and Sorgeloos, 1992; Knaeur and Southgate, 1997). Specialized equipment and facilities required for microalgae culture contribute to the cost of the hatchery. The development of suitable microalgae diet would be a major consideration to reduce the operational cost of a hatchery. Several alternative diets proposed are preserved microalgae, bacteria and yeast, microencapsulated and microbound diets, kaolin and silt (Knauer and Southgate, 1999). The most interesting alternative is refrigerated concentrated microalgae (Ponis *et al.*, 2003). The nutritional value of *Pavlova lutheri* can be maintained for 27 days when preserved at 1-4°C. The efficiency of this storage is because alga will remain as living cells when preserved at low temperature. *Tetraselmis suecica* after 3 months of storage, will still contained level of EPA equivalent to fresh alga (Montaini *et al.*, 1995). Provision of suitable ration will ensure the success of mollusc hatchery. According to Doroudi *et al.* (1999), food ration at 20×10^3 cells/mL for the larvae of *Pinctada margaritifera* resulted in the 230 µm anterior posterior length of larvae. While maximum survival of 8% when fed at 10×10^3 cells/mL. Even though growth is a major factor for the evaluation of physiological condition, survival percentage is still important at different culture density.

Algae ration and larvae density influence the growth and survival of larvae. The optimum algae ration varies depending on the larvae age. D-stage larvae showed high survival and shell grow when fed with microalgae at 4.5 – 11.5×10^3 cell/mL, at larvae density of

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$3mL^{-1}$ (Doroudi and Southgate, 2000). Larvae of 13- 20 day old has maximum survival when fed with microalgae at 2.5×10^3 cell/mL. Best shell growth of older larvae is when fed at 15- 32×10^3 cell/mL of microalgae. Doroudi and Southgate (2000) suggested the feeding of *P. margaritifera* at 8×10^3 cell/mL, with density of 3 larvae/mL up to 8-day old. While for 13 to 20 day old larvae, feeding at 25×10^3 cell/mL, with stocking of less than 2 larvae/mL. Therefore, the concentration of microalgae during culture affects the growth and survival of larvae (Doroudi and Southgate, 2000). Since bivalves are filter feeders, they require sufficient food to continue living. Concentration of microalgae to be fed to larvae varies depending on species of microalgae and larval stage. The minimum requirement of microalgae for *Crassostrea gigas* larvae is 25×10^3 cell/mL at D-stage (Rico-Villa *et al.*, 2006).

Fundamentally, microalgae culture is important to commercial hatchery of marine molluscs. According to Martinez-Fernandez *et al.* (2006), the used of *Pavlova* sp. and *Pavlova salina* in diet resulted in the highest shell grow at D- stage and umbo stage larvae for *Pinctada margaritifera. Pavlova* sp. and *Isochrysis* sp. from the group Prymnesiophytes are the most preferred due to their size and shape which facilitated better ingestion. Culture of *S. echinata* has been quite successful using diet composed of T-ISO and *P. salina* (Southgate and Lee, 1998). Martínez-Fernández and Southgate (2007) indicated the possibility of combining seven tropical microalgae as feed for D-stage larvae of *Pinctada margaritifera*, with the addition of diatom at umbo-stage larvae. Feeding with *Pavlova* sp. supports better growth of D-stage larvae, and with the addition of diatom it can increase the growth of umbo-stage larvae. Thus, suggesting that *Pavlova* sp. and *Pavlova* sp./*Chaetoceros muelleri* are good diet for oyster larvae.

Diet composition may affect the growth and survival of bivalve larvae. Various algae ration has been used in the oyster larviculture. A mixture of microalgae species will provide a better nutrient balance (Ronquillo *et al.*, 2012). Monospecific diet using *Tetraselmis suecica* resulted in the lowest growth of mangrove oyster, *Crassostrea corteziensis* due to the lack of DHA and low level of Arachidonic acid (20:4(n-6), AA) and EPA (Rivero-Rodriguez *et al.*, 2007). Arachidonic acid (AA) also is a fatty acid essential for the growth of juvenile clam *Tapes* sp. (Caers *et al.*, 1999). Ratio of EPA/DHA affects the growth of mollusc. Juvenile oysters fed with microalgae with high levels of essential fatty acid (EPA, DHA, AA) showed better growth compared to those containing lower level of fatty acid (Ronquillo *et al.*, 2012). The eye-larvae of *C. iredalei* showed better setting rate when fed with both *Isochrysis galbana* and *Chaetoceros calcitrans.* Meanwhile, larvae given *I. galbana* alone produce better results than *C. calcitran* alone. The best

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density of microalgae is 100×10^3 cell/mL for *I. galbana*, *C. calcitran* and mixture of both microalgae.

Conclusion

The successful production of oyster in hatchery depend highly on microalgae culture. However, selection of suitable microalgae should also be given priority. Other factors that may affect the production of oysters in hatchery are quality of broodstock, spawning technique, stocking density and water quality. Economic aspect should be considered as well, since low cost method will definitely reduce the overall hatchery operational cost. Development of culture technique for native oyster species will be more feasible since it may reduce obstacles in term of species adaptability to the local climate, and the availability of broodstock from the wild. Thus, in Malaysia, *C. iredalei* and *C. belcheri* have high potential as candidate for aquaculture.

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