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**วงจรการสืบพันธุ์ของหอยมุกเกลบ *Pinctada fucata* (Gould)**  
**Reproductive Cycle of the Pearl Oyster, *Pinctada fucata* (Gould)**

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### บทคัดย่อ

กระบวนการผลิตมุกโดยการฝังแกนมุกเข้าไปในอวัยวะสร้างเซลล์สืบพันธุ์ของหอยมุกเกลบ *Pinctada fucata* (Gould) เป็นขั้นตอนที่ต้องอาศัยความชำนาญและเป็นต้นทุนสำคัญจึงจำเป็นต้องหาวิธีการที่สะดวกและปลอดภัยต่อหอยมุกในการคัดเลือกขนาดของหอยมุกที่มีอวัยวะสร้างเซลล์สืบพันธุ์ที่พร้อมสำหรับการนำมาฝังแกนมุก เพื่อหลีกเลี่ยงการบาดเจ็บจากการผ่าตัดจริง จึงทำการศึกษาหอยมุกตั้งแต่แรกเกิดจนอายุ 12 เดือน เก็บตัวอย่างทุกเดือนๆ ละ 40 ตัว แล้วศึกษาวิทยาเนื้อเยื่อของอวัยวะสร้างเซลล์สืบพันธุ์ระยะต่างๆ ในวงจรการสืบพันธุ์ตลอดระยะเวลาการเจริญเติบโตวัดค่าเฉลี่ยของความยาวและความสูงเปลือกพร้อมทั้งหาปริมาณอินทรีย์สารคือ ไขมัน โปรตีนและไกลโคเจนของตัวหอย พบว่าหอยมุกเกลบช่วงอายุประมาณ 11 เดือนซึ่งมีขนาดความยาวเปลือกเฉลี่ย  $64.84 \pm 3.1$  มม และความสูงเปลือกเฉลี่ย  $58.66 \pm 1.9$  มม มีอวัยวะสร้างเซลล์สืบพันธุ์ที่เหมาะสมสำหรับการนำมาผ่าตัดฝังแกนมุกเป็นครั้งแรก

**คำสำคัญ :** วงจรการสืบพันธุ์ของหอยมุก หอยมุก หอยมุกเกลบ การใส่แกนมุก

### Abstract

The process of nucleus insertion into gonad of the pearl oysters, *Pinctada fucata* (Gould) is the major procedure requiring skill and economy. In order to survive the scarce oysters, the conveniently selection of the oysters with proper gonad for nucleation was studied. Each 40 cultured Akoya pearl oysters were sampling monthly for 12 months. Average measurement of shell length and shell height were taken place around 12 months of development. Every developing oyster was studied the histological structure of gonad with various stages of reproductive cycle. In addition, the monthly increasing of the organic composition of mantle: protein, Lipid and glycogen was calculated. The result of proper gonadic stage for first nucleation appeared on the 11 month-old oysters, which their average shell length equaled to  $64.84 \pm 3.1$  mm and average shell height equaled to  $58.66 \pm 1.9$  mm By this stage, the gonad belonged to spawning and early spent stage .

**Keywords :** reproductive cycle, pearl oysters, *Pinctada fucata*, nucleus insertion

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## Introduction

The Akoya pearl oysters are common species along Andaman Coast and can be bred in coastal Phuket (Kanjanchatree *et al.*, 2004). Akoya pearl production took 2-3 years long and the survival rate of the pearl oysters after nucleus insertion was so low of about 29-32% (Kanjanchatree *et al.*, 2006). The problems due to such as misplace nucleus insertion and pre-, post-dissection management that stressed metabolism of the oysters. Moreover, slightly understanding about the stages of gonadic development of the Akoya pearl oysters is the most important factor. Accordingly, the gonad is the appropriate site for nucleus insertion. Therefore the proper reproductive stage for nucleus insertion is the spawning stage, since the insertion taken place in the gonad of mature stage can cause nucleus extrusion (Meng *et al.* 1994). This study aimed to determine gonadic stages of the first reproductive cycle of Akoya pearl oysters (*Pinctada fucata*) cultured from earlier age to 12 months old (May 2005 to April 2006). In addition, the oysters' shell length and shell height were recorded throughout their development. These data can be used to determine the suitable body-size of the oysters that have appropriate gonadic stage for the first nucleus insertion without dissection the oysters. Since that activity for checking their gonad before nucleus insertion, will lead to their mortal hazard. Finally, the farmers can easily collect the oysters with suitable gonad for nucleus insertion, so the productivity is increased and moreover, the cost of pearl culturing is lower.

## Materials and methods

### 1. Breeding of the Akoya pearl oysters

#### 1.1 Preparation of the oysters' parents for fertilization

The oysters' parents having shell length more than 60-65 mm were collected. After their fecundity of the gonad was microscopic checking. Male and female oysters

were separated. by temperature shock method (Kanjanchatree *et al.*, 2004).

#### 1.2 Oyster culture

The fertilized eggs reared in culturing tank were going to cleavage and differentiate to D-shape larvae. Later, larvae were fed with *Isochrysis* sp. and *Chaetoceros* sp. until they developed to Eyed larvae. (Kanjanchatree *et al.*, 2004). Then, the spats were rearing in the sea at 1 m depth. (Kanjanchatree *et al.*, 2003). The 40 oysters were randomly collected monthly from the age of 1 month to 12 months, and measured their shell lengths and shell heights. In addition, the temperature, salinity, oxygen concentration and pH of the sea water were recorded monthly.

### 2. Histological study

Histological structures of every oyster's gonad were examined the gametogenic stages by standard histological technique. Developmental stages of the oysters' gonad are classified into six stages modified from the criteria used by Wada *et al.* (1995) and Choi and Chang (2002).

3. Quantitative analysis of organic composition of the adductor muscle along 12 months of development Lipid analysis by C-M mixture technique were undertaken. (Drouillard *et al.*, 2004). Protein and Glycogen analysis were resolved by Lowey's technique (Lowey *et al.*, 1969).

## Results

### 1. Growth and organic composition of oyster

Growth of the Akoya pearl oyster, their average shell lengths and shell heights were continuously increased. Simultaneously, their accumulation of protein, lipid and glycogen were fluctuated increment. (Table 1)

### 2. Reproductive cycle

Akoya pearl oysters are sexual dimorphism, However, 2 oysters of the total 360 individuals were hermaphrodite which corresponded to the study in Japanese Akoya pearl oysters, *Pinctada fucata fucata* by Wada *et al.* (1995).

**Table 1.** Organic composition of adductor and average size of the pearl oysters, *Pinctada fucata* throughout the year.

Age (Months)	Protein (%)	Lipid (%)	Glycogen (%)	Shell (mm)	
				length	height
1	1.97	0.22	0.23	12.45 ± 1.6	10.95 ± 0.9
2	3.21	0.26	0.3	18.60 ± 2.3	16.88 ± 2.0
3	3.87	0.33	0.53	22.86 ± 2.1	18.25 ± 0.7
4	4.11	0.38	0.89	26.79 ± 1.6	21.03 ± 2.0
5	6.37	0.59	1.18	30.79 ± 2.2	24.53 ± 1.1
6	9.52	0.81	1.34	33.65 ± 2.5	27.81 ± 2.2
7	9.81	0.9	1.57	38.48 ± 2.2	31.83 ± 1.8
8	10.22	1.07	1.62	44.88 ± 3.5	36.70 ± 2.2
9	10.45	1.15	1.82	52.76 ± 3.6	44.46 ± 4.5
10	10.77	1.26	2.14	57.37 ± 3.6	50.49 ± 1.6
11	10.03	1.33	2.46	64.84 ± 3.1	58.66 ± 1.9
12	11.11	1.41	2.73	71.50 ± 4.8	63.68 ± 3.1

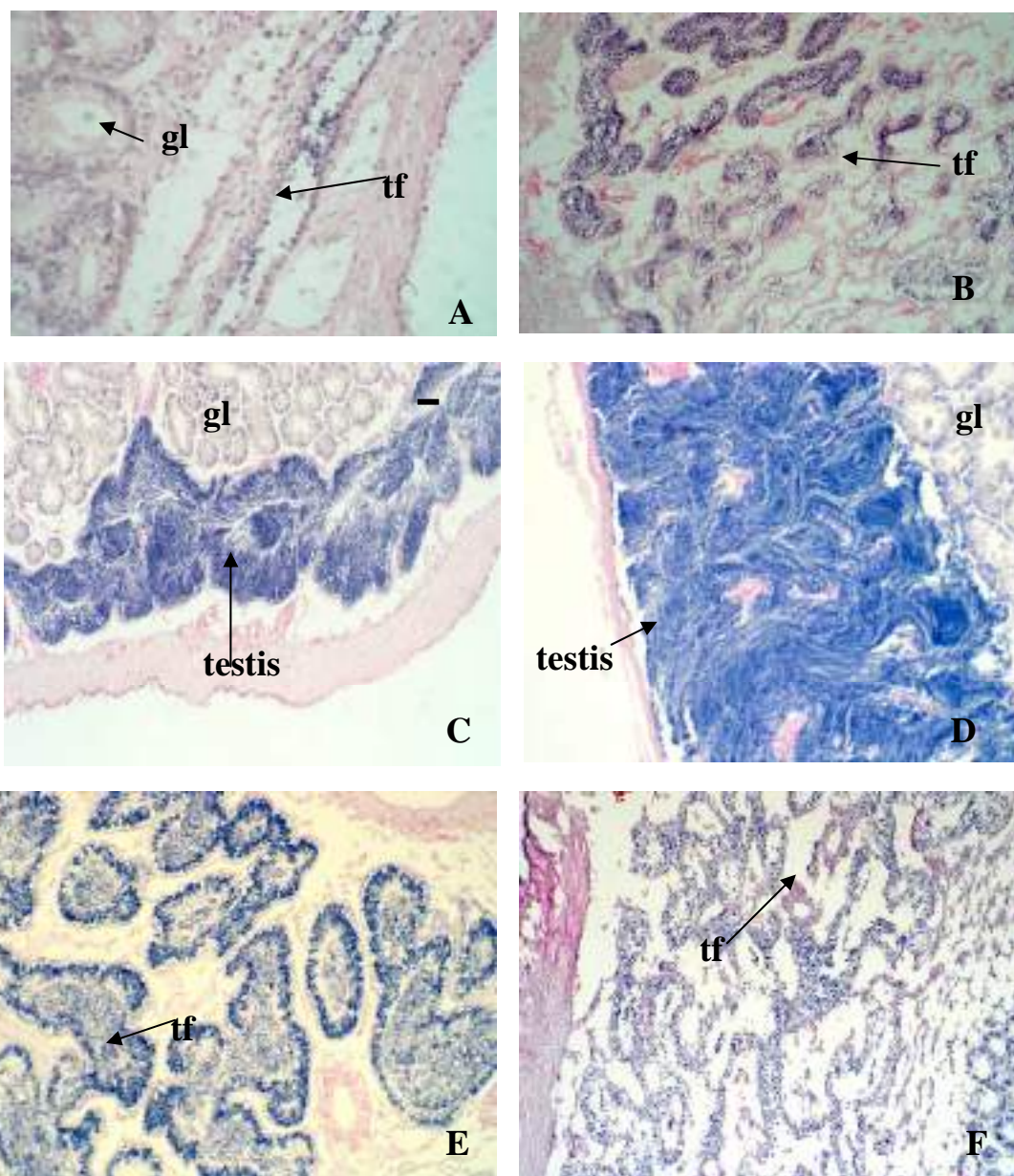
They found 2 hermaphrodite oysters from the total 232 individuals. The gonad of hermaphrodite oyster filled with sperms and oocytes in the same follicle. Histological examination of the 6 reproductive stages was described below.

1. Initial developing stage : Gametogenesis was first appeared in 4 month-old oysters (Figure 1A, 2A). Every oyster had developing gonad with small follicles surrounded by numerous connective tissue. The gonad follicles contained small stem cells lining germinal epithelium of the follicles. The distinguishable males had the follicles lined with spermatogonia of about 3-4 m diameters. The females had oogonia of about 4-5 m diameters along some parts of follicle wall.

2. Multiplicative stage : This stage was first appeared in 5 month-old male oysters (Figure 1B ), while in female oysters were 6 months old (October 2005) (Figure 2B). The more developing gonad contained numerous follicles yet among with much connective tissue. Gonad follicles contained oogenic and spermatogenic cells which

proceeded to proliferate into different stages of development. Spermatocytes in the testis were small cells with dark blue nuclei. In the ovary, small oocytes with basophilic cytoplasm were proliferating along the wall of follicle.

3. Growing stage : The expanded gonad follicles were filled with different stages of developing gametes. Gamete formation proceeded actively in the follicles among with decreasing coonective tissue. This stage was first appeared in 6 month-old male oysters (Figure 1C), while in female oysters were 7 months old (Figure 2C ). The enlarging dark -blue testis in which numerous developing spermatocytes and spermatids were evenly distributed in the follicular lumen. The enlarging ovary in which various stages of developing oocytes were proceeded expanding into the follicular lumen. Primary oocytes along the follicular wall had diameters about 30 µm. Newly mature oocytes could be found at the center of the follicle. These are the large polygonal cells having large nuclei and cytoplasm with accumulating yolk.



**Figure 1.** Histological structures of testicular follicles of *Pinctada fucata* showing spermatogenesis during 12 months of the pearl oyster's development. (H&E), (A-B 400, C-F 100)

A = initial developing stage, tf = testicular follicle, gl = digestive gland

B = multiplicative stage

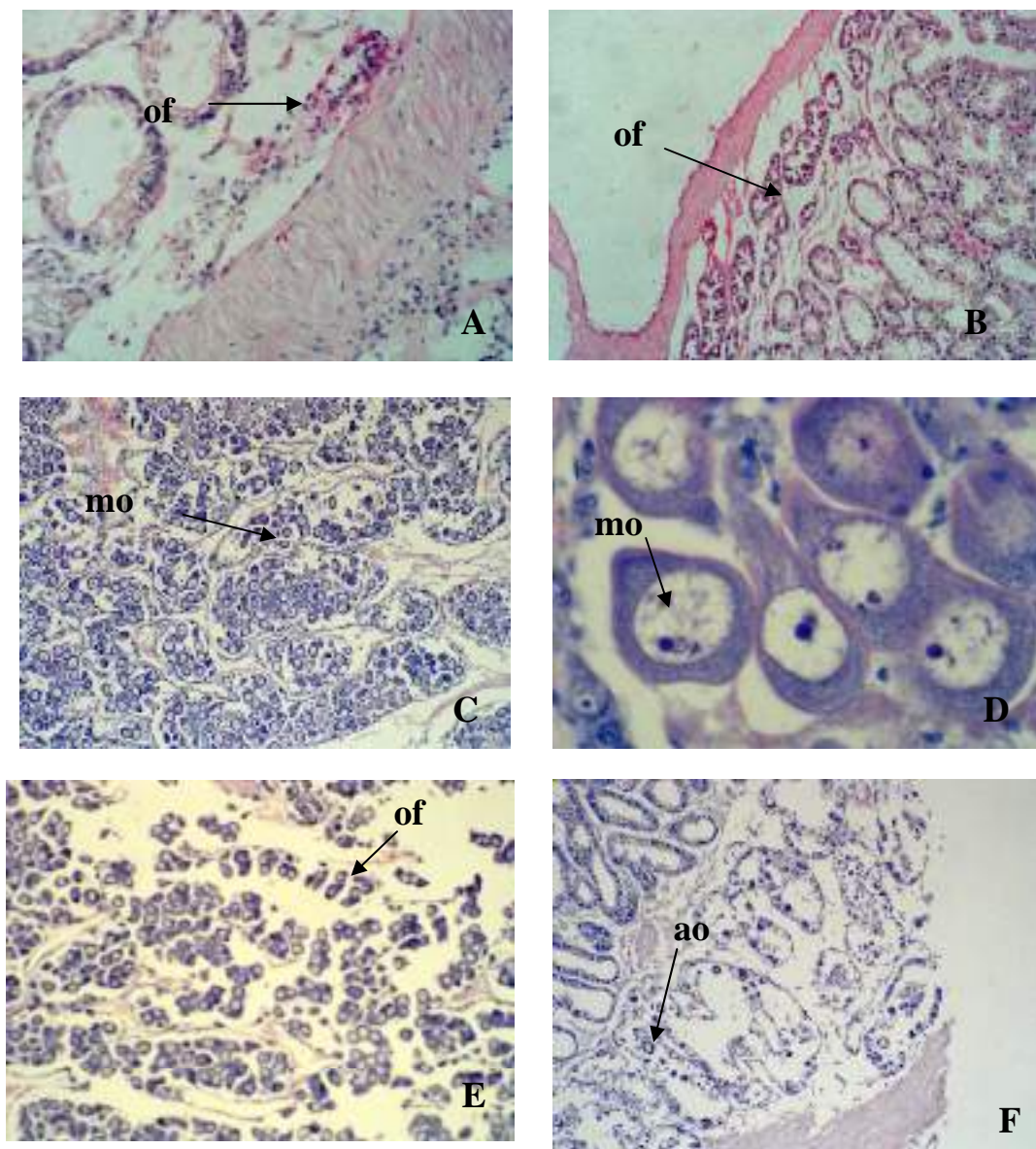
C = growing stage, numerous spermatocytes with dark blue nuclei and spermatids distributed in the lumen of testicular follicles.

D = mature stage, the follicles filled with different stages of spermatocytes (dark blue color)

E = spawning stage

F = resting stage or spent stage





**Figure 2.** Histological structures of the ovary of *Pinctada fucata* showing ovarian follicles with oogenesis during 12 months of the pearl oyster's development. (H&E), (A, B, E 100, C, F 400, D 1000)

A = initial developing stage, of = ovarian follicle

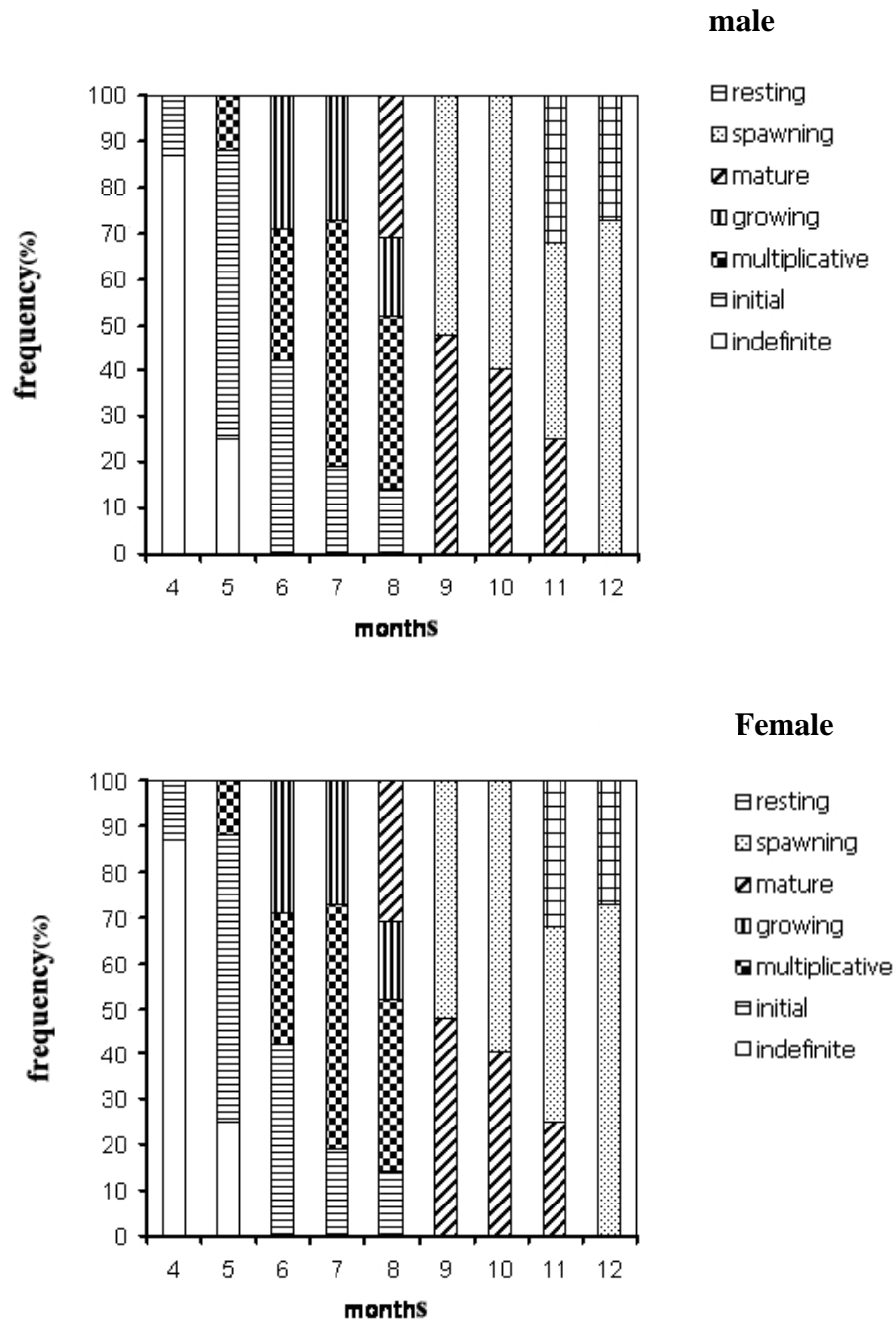
B = multiplicative stage

C = growing stage, mo = mature oocyte

D = mature stage, large mature oocytes with distinct nuclei and nucleoli

E = spawning stage

F = resting stage, ao = atretic oocyte with shrinkage cytoplasm



**Figure 3.** Monthly changes of frequencies of gametogenic stages (%) of the total individuals of pearl oysters, *Pinctada fucata*.

4. Mature stage: Ripe gonad follicles, surrounding with a small thin layer of connective tissue, were densely packed with maturing gametes. This stage was first appeared in 8 month-old male oysters (Figure 1D), while in female oysters were 9 months old (Figure 2D). The largest testis was fully filled with dark blue spermatocytes, especially spermatozoa in center of the follicle. The ovary was packed with a large number of mature oocytes which appeared freely in the follicular lumen. The oocytes are polygonal shape and some are spherical with diameter about 40-45  $\mu\text{m}$ . However, some oocytes are stalk shape, having cytoplasmic process attached to the follicular wall.

5. Spawning stage : This stage was first appeared in 9<sup>th</sup> month of development (Figure 3) and continuously spawned until 12<sup>th</sup> month. In the male oyster (Figure 1E): most spermatozoa were released, some follicles were partially empty, in which immature spermatocytes or spermatids were restricted to the lining of the follicular wall. In the female oyster (Figure 2E): gonad follicles were distended, most of ripe oocytes developed free from the follicular wall and move into the center, and then were released. At the same time, a few ripe oocytes remained and follicles become looser.

6. Resting stage or spent stage : This stage was first appeared in 11<sup>th</sup> month of development and continuously spent until 12<sup>th</sup> month). Follicles were almost empty or completely spawned (Figure 3). The testis (Figure 1F) or the ovary (Figure 2F) become contracted, in which the follicular lumen had residual spermatozoa or oocytes and showed signs of regression. Some shrunk follicles had stem cells along follicular wall. Therefore, this stage was preparing the reconstruction of new follicular tissue of the next reproductive cycle. The intervened connective tissue had been increased. Growth of shell sizes and organic composition.

## Discussion and Conclusion

1. The 4 month-old Akoya pearl oysters were 85% gonadic indefiniteness, while 15% of them had definite

sex contributed to the initial stage of gametogenesis. The 5 month-old oysters had 22.5% of indefinite gonad, while being 67.5% of initial stage and the rest 10.0% being multiplicative stage.

2. The procedure of culturing pearl oysters for spherical pearl production, the most suitable site for nucleus insertion is their gonad. Because the appropriate gametogenic stage for easily nucleation is the period of late spawning stage to spent stage, in which the gonad follicles are empty. Hence, the 11 month-old Akoya pearl oysters were the best situation for the first nucleus insertion. Simultaneously, during development of the oysters, their shell sizes were continuous increased. As a result, the proper pearl oysters with average shell length of  $64.84 \pm 3.1$  mm and average shell height of  $58.66 \pm 1.9$  mm are being the first harvested for nucleation. Easefully, the farmers can harvest the pearl oysters which have shell length more than 64 mm or shell height more than 58 mm for the first nucleation. By this knowledge, it decreases the oyster's injury from conventionally random dissecting check was decreased.

3. Along 8 months of the pearl oyster development, they mostly accumulated protein much more than lipid and glycogen (Figure 6). However, the highest accumulation of Lipid was on the 8<sup>th</sup> month of development, in contrast to lowest glycogen accumulation. This reflected body growth and gonad development. On the other hand, the 9-12 month-old oysters continuously increased glycogen accumulation, which was more than protein and Lipid. This implied that the spawning process required enormous energy (Saucedo *et al.*, 2001).

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