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Early embryonic developmental stages of the pearl oyster *Pinctada radiata* (Leach, 1814) from Lattakia coast, Syria

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Abstract - Matured brood stock of *Pinctada radiata* were collected from Afamia region in Lattakia coast. Three sets of spawning and larval rearing experiments were conducted during the period from July - October 2016 and the pearl oysters were successfully spawned at average temperature 31.5 ± 0.26 °C. After about 60 min, oocytes presented no germinal vesicle, rounded and measured 47.5-55 µm with an average of 50 ± 3.95 µm in diameter. Early stages of embryonic development were recorded and the polar body was observed 15 min after fertilization, trefoil stage at 1 h and 30 min, morula at 3 h and 30 min, blastula at 4 h and 50 min, gastrula at 6 h, trochophore larva at 8 h and veliger larva at 20 h. Veliger larva dimensions were 61.88 ± 3.82 µm for average length along the antero-posterior axis and 51.26 ± 5.14 µm for height along the dorso-ventral axis.

Key words: Hatching, Veliger larva, Embryonic development, Pearl oyster, *Pinctada radiata*.

Introduction

Pearls are one of the first jewels discovered by humans for thousands of years. Since then, people from different cultures and civilizations have recognized the beauty and value of pearls. Pearls are the only organic jewels that need no treatment to reveal their natural beauty. In the beginning, man depended to find these natural pearls on a variety of marine bivalves and fresh water mussels. Natural pearls are rare, and perhaps 1 out of 2,000 pearl oysters can contain natural pearls (Haws, 2002).

Pearl farming offers a significant and important contribution to the economic development of communities, especially in coastal villages through the most valuable species. The pearl industry does not require large amounts of money, but the limit will be sufficient. It also offers many benefits to workers in aquaculture farms, coastal communities and national economies. Pearls are ideal commodities for export, they are non-perishable, shipping costs are negligible, and their markets are at the same time profitable (Gervis and Sims, 1992).

Before starting the culturing and farming of pearl oyster or another bivalves, a set of biological characteristics must be studied such as spawning and larval development.

There are many studies that dealt with bivalves such as the larval development (Chanley, 1965), rearing and farming (Loosanoff and Davis, 1963), induced spawning and larvae growth (Wong *et al*., 1986; Nayar *et al*., 1984), production of spat (Alagarswami *et al*., 1983), spawning and larval rearing (Sreenivasan and Rao, 1991; Narasimham *et al*., 1988; Muthiah *et al*., 1992), reproductive, age and growth (Jagadis and Rajagopal 2007 a; b).

This research aimed to study the induced of spawning by thermal stimulation, early embryonic development and rates of fertilization and hatching for *P. radiata* under laboratory conditions.

Materials and Methods

Mature pearl oysters *P. radiata* were collected from Afamia region in Lattakia coast, Syria (latitude 35° 32' 47" N and longitude 35° 45' 21" E) (Fig. 1), manually at low tide from the littoral zone, during July, August and September 2016, and were transported to the laboratory, Department of Zoology, Faculty of Science, Tishreen University.

Figure 1. Location of the sampling area (Afamia region in Lattakia coast) taken by Google Earth.

The Shell Height (SH) or Dorsoventral Measurement (DVM), Shell Length (SL) or Anteroposterior Measurement (APM), Hinge Length (HL) of the pearl oyster *P. radiata* were measured with a Vernier caliper to the nearest 0.1 mm (Fig. 2), while wet weight (TW) was measured using a digital balance to the nearest 0.0001 g (Lodola *et al.*, 2013).

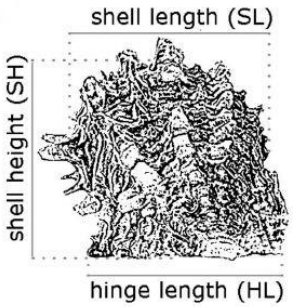

Figure 2. Morphological measurements of *P. radiata* shell carried out on the left valve (the largest valve).

The veliger larva dimensions were measured through to the antero-posterior and dorso-ventral axes using the ocular micrometric (Fig. 3), and correlation relationship between these dimensions were calculated.

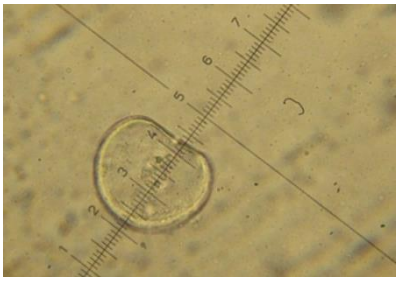

Figure 3. The veliger larva of *P. radiata* from a lab. hatchings.

The pearl oysters were placed in two plastic tanks (50 x 30 x 25 cm) containing filtered seawater by biological filter (consists of: cotton, active carbon, active ceramics, Ultra-Violet light and biological balls) and equipped with oxygen pumps. Three sets of spawning and larval rearing experiments were conducted during the period from July to October 2016 (Table 1).

After conditioning for ten days, twenty individuals were used for spawning experiment. They were put in 30 liter plastic tanks, the temperature was slowly raised above the normal limit by adding hot seawater or by thermostat controlled heating element (Table 1).

The tanks were covered with plastic cover to prevent light and dust. Samples were collected every 15 minutes and observed till the eggs transformed into veliger or D larva stage (Jagadis, 2011).

Data were analyzed based on the fertilization and hatching rates which were determined by taken eggs samples in 1 ml from each experiment with 3 replicates (Azuadi *et al.*, 2013):

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs in 1 ml}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs in 1 ml}} \times 100$$

Table 1. Dates and some environmental parameters of the three experiments conducted in the laboratory.

experiment	Environmental parameters			
	Temperature °C	Salinity ‰	pH	Dissolved Oxygen mg/l
1/7 - 1/8/2016	25.7	38.1	7.84	4.86
12/8 - 12/9/2016	27.1	37.5	8.11	4.77
27/9 - 27/10/2016	26.6	38.9	7.92	4.62

Results

A total of 60 *P. radiata* males and females individuals (distinguished by size, males smaller than females) were sampled for the three experiments, individuals were directly detached manually from the substratum as beached individuals.

SH values for the individuals sampled ranged between 34.1-65.8 mm, SL ranged between 30.4-57.7 mm, HL ranged between 31.1-45.2 mm and TW ranged between 5.1468-27.3119 g). The mean values (± Standard Deviation) for SH, SL, HL and TW were recorded (Table 2).

Table 2. Mean values (±SD) of the morphological measurements of the adult *P. radiata*.

Experiments		Gender	N	SH ± SD	SL ± SD	HL ± SD	TW ± SD
1	1/7/2016	F	10	49.1 ± 3.1	45.9 ± 3.5	42.8 ± 2.5	13.7452 ± 3.3030
		M	10	36.6 ± 2.1	34.5 ± 3.8	36.6 ± 3.8	6.7535 ± 1.3649
2	12/8/2016	F	10	50.2 ± 6.4	45.5 ± 6.5	40.3 ± 3.5	14.4964 ± 6.0216
		M	10	37.3 ± 2.4	35.0 ± 2.6	37.0 ± 3.6	6.9590 ± 1.1675
3	27/9/2016	F	10	52.5 ±7.3	49.7 ± 5.2	43.5 ± 3.0	16.4887 ± 6.1975
		M	10	37.1 ± 2.3	36.5 ± 4.2	36.6 ± 3.8	7.1128 ± 1.3105

The spawning of *P. radiata* was induced and carried out in three experiments. The results showed that an increased of ambient temperature to 4.5-6 °C (mean ± SD: 5.2 ± 0.75) was effective to induce spwining. In all attempts, males first released sperms, then the females laid the eggs afterwards. The release of the gametes by one of the oysters leads to the spawning of the other. The spawning lasted from 45 - 60 minutes and the water became milky because of the persistent release of the sperms and eggs. The fertilization rate ranged between 87.5-95.5 % with a mean of 92.3 ± 4.25, the hatching rate ranged between 80.6-87.4 % with a mean of 84.4 ± 3.46. (Table 3). Mother oysters have been removed from the tanks to avoid any water filtration process done for feeding, which may affect the number of eggs (Fig. 4).

Table 3. Temperature, fertilization and hatching rates during induced spawning of *P. radiata*

Experiment		Data				
		Ambient Temperature $°C_1$	Spawning Temperature $°C_2$	Amount of Increase $°C_2 - °C_1$	Fertilization rate (%)	Hatching rate (%)
1	1/7-1/8/2016	25.2	31.2	6	87.5	80.6
2	12/8-12/9/2016	27.1	31.6	4.5	95.5	87.4
3	27/9-27/10/2016	26.6	31.7	5.1	94	85.1
Mean ± SD		26.3 ± 0.98	31.5 ± 0.26	5.2 ± 0.75	92.3 ± 4.25	84.4 ± 3.46

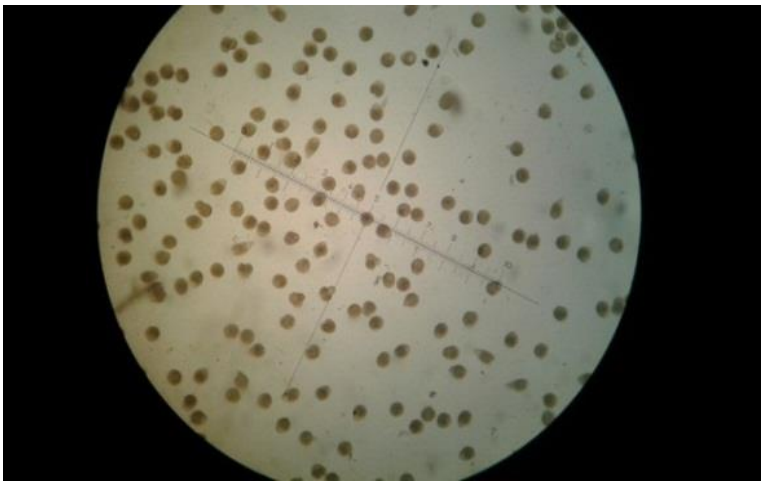

Figure 4. Eggs of the pearl oyster *P. radiata*.

Thirteen stages were recorded from the non-fertilized oocytes or immature oocytes to the veliger larvae or "D" shaped larvae (Figs. 5 & 6), in addition to the timing of each stage after fertilization (Table 4).

Immature oocytes appeared in “pyriform” shape and were released immediately after the males released sperms (Fig. 5-A), after 30-50 minutes, immaturity of the oocytes were shown by the presence of a germinal vesicle (Fig. 5-B), after about 60 min, oocytes presented no germinal vesicle, rounded and measured 47.5-55 µm with a mean of 50 ± 3.95 µm, and were ready for fertilization (Fig. 5-C).

The first polar body appeared after 15 minutes of fertilization (Fig. 6-D) and the cleavage started:

- 2-celled stage:
  The first cell division is seen 45 minutes after fertilization and led to the formation of a micromere (mic) and a macromere (mac) (Fig. 6-E).
- Trefoil stage:
  During the second cleavage the micromere part is divided into two equal parts, while the macromere is divided unequally or unevenly into a micromere and macromere. The stage with three micromeres and a macromere is called Trefoil stage (Fig. 6-F).
- Morula stage:
  The macromere part is not divided into more divisions. Micromeres are divided several times and repeated to become smaller and smaller and passes through 8 cells, then 16 cells, and so on until it reaches the morula stage (Fig. 6-I).
- Blastula stage:
  The embryo became spherical with transparent cells and a blastocoel. A cell redirection begins and the blastocoel and blastopore are formed (Fig. 6-J).
- Gastrula stage:
  The gastrula is formed by the early movement of cells that occur in the embryo. The embryo with minute cilia looks like a bean because of cell convolution (Fig .6-K).

- Trochophore larva stage:
  A single apical flagellum is developed at the anterior side. The anterior portion of the larva is broader while the posterior end is spiked like an inverted triangle. The minute cilia that have formed in the gastrula stage disappear (Fig. 6-L).
- Veliger larva stage:
  The larvae acquired a definite D-shape by producing the primary embryonic shell (prodissoconch I) with the appearance of the hinge line (Fig. 6-M). The veliger larva measures 61.88 ± 3.82 µm as APM (along the antero-posterior axis) and 51.26 ± 5.14 µm as DVM (along the dorso-ventral axis), the correlation relationship between APM and DVM gave the equation:

$$APM = 0.6661\ DVM + 27.737$$

  and the correlation coefficient was R= 0.98 (Fig. 7). This indicates that the relationship is positive and reliable.

Table 4. Timing of early embryonic development stages after fertilization of *P. radiata*.

Stage	Time (minutes – hours)
Immature Pyriform oocyte	0
Immature oocyte with a germinal vesicle	0
Fertilized egg	0
Fertilized egg with a polar body	15 min
2-celled	45 min
Trefoil stage	1 h and 30 min
8-celled	2 h and 45 min
16-celled	3 h
Morula	3 h and 30 min
Blastula	5 h and 50 min
Gastrula	9 h
Trochophore larva	12 h
Veliger larva	20 h

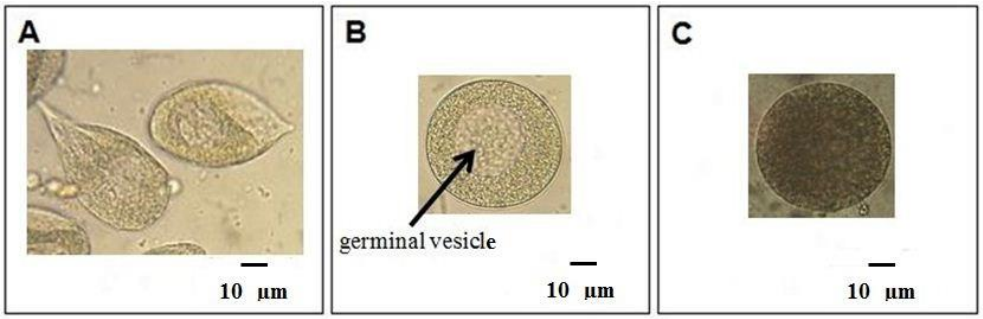

Figure 5. (A) Immature pyriform oocytes, (B) immature oocyte with a germinal vesicle, (C) fertilized egg.

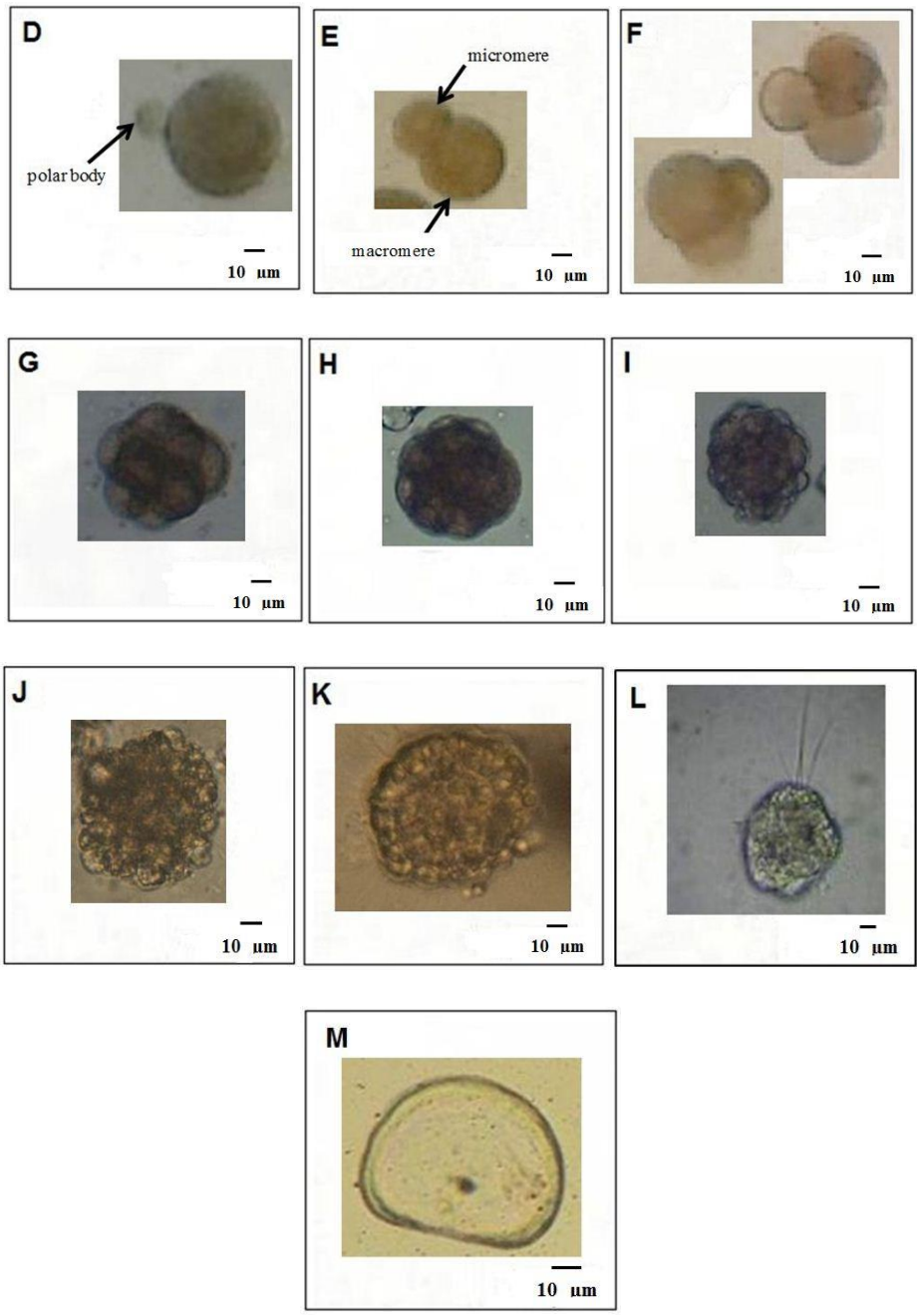

Figure 6. Early embryonic development of *p. radiata*, (D) Fertilized eggs with a polar body, (E) 2-celled stage, (F) Trefoil stage, (G) 8-celled stage, (H) 16-celled stage, (I) Morula stage, (J) Blastula stage, (K) Gastrula stage, (L) Trochophore larva stage, (M) Veliger larva stage.

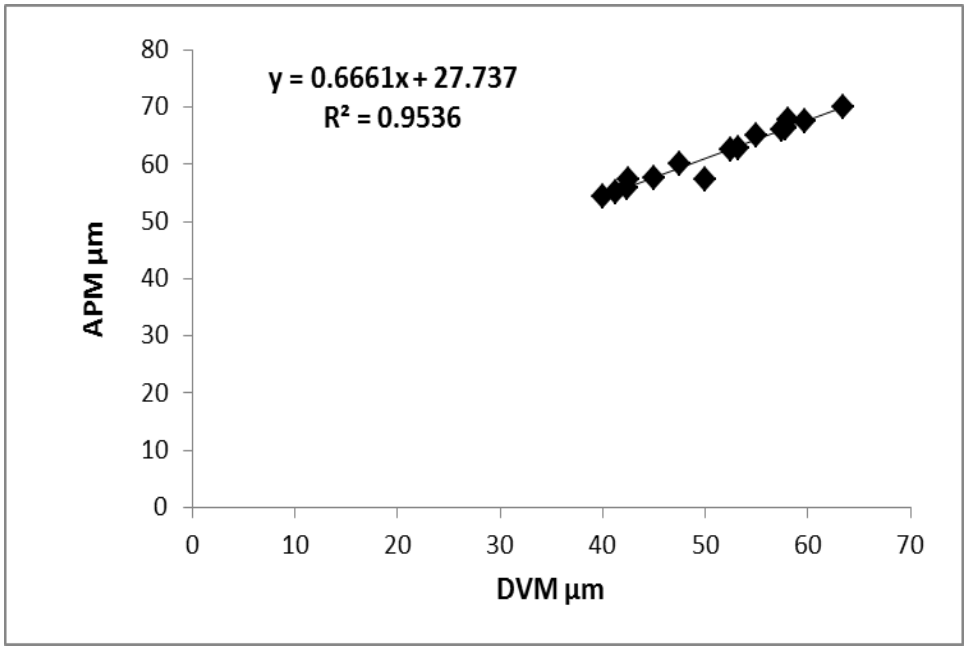

Figure 7: The relation between dimensions of veliger larval stage of *P. radiate*, APM= 61.88 ± 3.82 µm, DVM= 51.26 ± 5.14 µm.

Discussion

The maximum size of SH recorded for the adult *P. radiata* individuals in the present study was 65.8 mm which is less than that recorded from other studies in Tunisia: 100.5 mm at Bizerta lagoon (Tlig-Zouari *et al*., 2009; 2010), 104.34 mm at Hammamet (Bellaaj-Zouari *et al*., 2012) and 96 mm in the Gulf of Gabes (Derbali *et al*., 2011).

Furthermore, in Egypt 64 mm was recorded as the maximum height that is a nearest value to the present results (Yassien *et al*., 2000). Differences in size might be due to various environmental factors, such as spatial competition with other species, predation, environmental pollution, lack of nutrients, irregular seasonal growth and reproduction activity (Lodola *et al*., 2013).

Usually, in such studies, the used marine water is filtered to exclude the small particles, as well as the treatment with UV to kill bacteria (Minaur, 1969; Walne, 1974), while in the present study, the marine water from Afamia region in Lattakia coast was hold to the laboratory and then filtered through the biological filter.

Spawning in bivalves, in general, is influenced by temperature changes (Alagarswami *et al*., 1984; 1987), it started with raising temperature 5 °C in summer and up to 10 °C in winter for *Venus striatula* (Ansell, 1961), for *Mercenaria mercenaria* the spawning was performed by raising temperature a few degrees (Loosanoff and Davis, 1963), and that agreed with the present study.

The thermal stimulation was done on many species of pearl oysters such as *P. maxima* where temperature was raised from 25.9 to 31.4 with an average 5.5 °C (Minaur, 1969; Tanaka and Kumeta, 1981), and this is in accordance with the

present results, the same for *P. fucata* and *P. margaritifera* where temperature was raised about 5 °C (Alagarswami *et al*., 1983; 1989).

It was found when comparing the average mature egg diameter in different species of bivalves that it was 62 µm for *Gafrarium tumidum* (Jagadis, 2011), and 75 um for *Meretrix meretrix* (Narasimham *et al*., 1988), 55 µm for *Anadara granosa* (Muthiah *et al.,* 1992), while for *P. fucata* it reached 47.5 µm (Alagarswami *et al*., 1983), the last two values were the closest to the present values.

Concerning early embryonic development, it was recorded for *P. maxima* that the beginning of first division was after 15 minutes from fertilization and the appearance of polar body, while the second division happened after 60 minutes, the morula stage was noticed after 3 hours, gastrula with the delicate cilia followed by trochophore larva was seen after 5 and 7 hours, respectively, while the veliger larva stage or D-larva, was noticed after 18-24 hours, where as the first embryonic shell (prodissoconch I) was formed after day 1 (Rose & Baker, 1994), which was in agreement with the result of the present study.

In the present study, the greatest value (67.76 µm) for the first embryonic shell length APM for prodissoconch I in D-larva, with average 61.88 ± 3.82 µm, while shell height DVM was 58.08 µm with average 51.26 ± 5.14 µm, and the correlation relationship between length and height was positive and reliable, for *P. maxima* the first embryonic shell dimensions were 79 um for shell length and 67 µm for height (Rose & Baker, 1994), while the dimensions for *P. fucata* were 72.5 µm for shell length and 57.5 µm for height (Alagarswami *et al*., 1983; Wada, 1984), in spite of that the larvae were from the same genus *Pinctada* sp. The differences in dimensions were visible, but all correlation relationships between shell length and height were positive and reliable and linear of the type y=ax+b (Loosanoff and Davis, 1963; Alagarswami *et al*., 1983; Narasimham *et al*., 1988; Muthiah *et al*., 1992).

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مراحل التطور الجنيني المبكر لمحار اللؤلؤ *Pinctada radiata* (Leach, 1814) من ساحل اللاذقية، سورية

نضال حسن و كاترين منصور و فائز صقر

قسم علم الحياة الحيوانية، كلية العلوم، جامعة تشرين، اللاذقية، سورية

المستخلص – جمع مخزون الأمهات البالغات من منطقة أفاميا من شاطئ اللاذقية، سورية. أجريت ثلاث تجارب للتفريخ وتربية اليرقات في الفترة الممتدة من تموز إلى تشرين الأول لعام 2016. تم بنجاح تفريخ محارات اللؤلؤ عند متوسط درجة حرارة بلغ 31.5 ± 0.26 م°، وبعد حوالي الـ 60 دقيقة ظهرت البويضات كروية الشكل ومن دون الحوصلة الجرثومية بقياس قطر 47.5 - 55 µm ومتوسط 50 ± 3.95 µm. سجّلت المراحل المبكرة من التطور الجنيني ولوحظ الجسم القطبي بعد 15 دقيقة من التلقيح، مرحلة ورقة البرسيم بعد ساعة و30 دقيقة، التوتي بعد 3 ساعات و30 دقيقة، الأريمه بعد 4 ساعات و 50 دقيقة، المعيدة بعد 6 ساعات،

اليرقة الدولابية بعد 8 ساعات واليرقة المبرقعة بعد 20 ساعة. بلغ متوسط طول اليرقة المبرقعة 61.88 ± 3.82 µm على امتداد المحور الأمامي الخلفي و 51.26 ± 5.14 µm للارتفاع على امتداد المحور الظهري البطني.

كلمات مفتاحيه: التفقيس، اليرقة المبرقعة، التطور الجنيني، محار اللؤلؤ *Pinctada radiate*.