REPRODUCTIVE CYCLES OF THE PEARL OYSTERS, PINCTADA FUCATA (GOULD) AND PINCTADA MARGARITIFERA (LINNAEUS) (BIVALVIA: PTERIIDAE) IN SOUTHWESTERN TAIWAN WATERS

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Acknowledgements
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REPRODUCTIVE CYCLES OF THE PEARL OYSTERS, PINCTADA FUCATA (GOULD) AND PINCTADA MARGARITIFERA (LINNAEUS) (BIVALVIA: PTERIIDAE) IN SOUTHWESTERN TAIWAN WATERS

Jiuan-Jiuan Hwang*

Key words: Pinctada fucata, Pinctada margaritifera, gonadal development, spawning, sex ratio, Taiwan.

ABSTRACT

The reproductive cycles of the pearl oysters Pinctada fucata (Gould) and Pinctada margaritifera (Linnaeus) were examined based on 339 and 284 specimens, collected in southwestern Taiwan. Except for the month of December 2002, Pinctada fucata were collected during the periods from March 2002 to September 2003. Pinctada margaritifera were collected over two periods: from March 2002 to November 2002 and from June 2003 to September 2003. Histological observations on gonadal development indicated that Pinctada fucata and Pinctada margaritifera were protandrous hermaphrodites, and both species exhibited different annual cyclical patterns, in which maturity peaked in May and October for Pinctada fucata, and in July and November for Pinctada margaritifera. Both species had two spawning periods a year. The onset of reproduction appears to be regulated by sea surface temperature. For both species, females outnumbered males during the spawning season.

INTRODUCTION

The pearl oysters, Pinctata fucata (Gould) and P. margaritifera (Linnaeus) inhabit temperate, subtropical and tropical coral reefs, and are widely distributed in the Indo-Pacific area, down to northern Australia throughout the equator extending up to the southernmost region of Japan. In Taiwan, P. fucata and P. margaritifera are distributed in Yenliau (Taipei County), Jukeng, Kenting (Pingtong County), Hugin (Penghu Archipelago) and Hualien [11, 13].

In 1893, half-pearls were created by Mikimoto in Japan; a decade later, round pearls were produced by Mise and Nishikawa [2, 12, 17]. Since then, a number of biological studies have been done on P. fucata (= martensii) in Japan. Pearl oysters draws research attention because their use in the commercial culturing of pearls can potentially provide economic benefits to the many countries where the resources are available. Japan has marketed and exported most of the world’s round pearls, products of P. fucata species. Australia and the Indo-Pacific countries, including French Polynesia, Cook Islands, and Indonesia produce most the world’s half-round (blister) pearls and a small part of the world’s round pearls, both products of P. margaritifera [28].

For pearl production, gonadal maturation is important to pearl production, because it is essential to the artificial seed production and the preoperative procedures of the nucleus implantation. A large number of studies on the spawning period of P. fucata were published in the 1950s in Japan [19, 31, 38, 39, 45], and on the spawning period of P. margaritifera [27]. In Australia, Tranter [33-37] described three Pinctada species: P. albina, P. fucata and P. margaritifera. The gonadal development, maturation and spawning of these oysters have also been well documented [1, 3, 16, 20, 27, 30, 32, 40-43, 45]. In Taiwan, although a diversity of pearl oysters of the family Pteriidae have been found [11], little is known about the biological aspects of the wild stocks of these species. Therefore, this study investigates the reproduction cycles of wild stocks of two species, P. fucata and P. margaritifera in the waters of southwestern Taiwan. The results obtained in this study can provide useful information for pearl culture in the future.

MATERIALS AND METHODS

In total, 339 and 284 specimens of the pearl oysters Pinctada fucata and P. margaritifera were collected on
a monthly basis from March 2002 to September 2003 (with the exception of December 2002), and from March 2002 to November 2002 and from June 2003 to September 2003, respectively, by divers at 3 to 25m-depth in the coastal waters of southwestern Taiwan (Figure 1). The sea surface temperature (SST) of sampling sites was also recorded monthly. Specimens of *Pinctada fucata* and *P. margaritifera* ranged in size: 40.4-71.0 mm and 54.1-137.4 mm, respectively (Table 1). The shell height (dorsoventral measurement) was measured with a vernier caliper to the nearest 0.1 mm. In the histological observation of the gonadal development, the tissue of both species was sampled from the end of gut loop of the visceral mass. These samples were fixed in a 10% formaldehyde solution for 24 hours, and were then, washed and dehydrated in a series of ethanol from 70 to 100%, embedded in paraffin, sectioned in 5µm thick, and stained with hematoxylin and eosin.

Gonadal development was determined by histological observation of specimen under a light microscope at the magnification of objective lenses ×20 and ×40 with an attached camera (video), following the procedures of Tranter [34, 35], Uemoto [39] and Wada et al. [42]. The schemes were identified by the size and density of various germ cells, which are formed from stem cells of gemetogenesis in the gonad.

**RESULTS**

1. Gonadal development

The gonadal development of both *Pinctada fucata* and *P. margaritifera* were no difference, can be categorized into six stages in males (Figure 2) as well as in females (Figure 3a-f), a few hermaphroditic individuals (Figure 3g-h) were also identified as follows:

*Stage 1:* Early developing stage

The follicle walls were thick and small primary oogonia and few larger developing oocytes were attached to the follicle wall. In males, the follicle walls as well as stem cells lining the follicle walls were developing. At the same time, differentiation of spermatogonia, even a few spermatocytes could be observed. (Figures 2a and 3a)

*Stage 2:* Developing stage

The oocytes, more developed and appeared like pear-shape, are still attached to the follicle wall in females. Similarly, spermatocytes and spermatids filled the follicle lumen, while the male follicle walls become thinner. Compared to the previous stage, spermatogonia attached to the follicle walls are fewer in number. (Figures 2b and 3b)

*Stage 3:* Mature stage

The follicle is full of mature, round and expanded oocytes, tightly connected to each other. A few oocytes are still attached to the wall by the stalk. In this stage, the follicle wall is swollen and indefinite. In males, there are larger quantities of mature spermatids. Free active tailed-spermatozoon are observed in the lumen central. (Figures 2c and 3c)

*Stage 4:* Spawning stage

The interstices between the oocytes are loosening with the release of ova. Free oocytes are spawning and the follicle wall is broken. Part of spermatozoa had been released and there are still active spermatozoa as well as spermatids remaining within the follicle, making the central lumen appear to be a little emptier. A few resorptive phagocytic cells have appeared. (Figures 2d and 3d)

*Stage 5:* Spent stage

Not all follicles are empty, because some free oocytes and some residual oocytes, which are small or not well-developed, remained. Some gametes remain unreleased in the lumen. Although phagocytes have cleared unreleased oocytes, spermatids and residual
Table 1a. Specimens of *Pinctada fucata* used in histological study

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Number</th>
<th>Shell height (mm)</th>
<th>Shell width (mm)</th>
<th>M</th>
<th>F</th>
<th>I</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 25, 2002</td>
<td>20</td>
<td>58.1 ~ 63.3</td>
<td>25.0 ~ 24.5</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Apr. 22</td>
<td>19</td>
<td>52.7 ~ 68.9</td>
<td>21.4 ~ 26.6</td>
<td>12</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>May 24</td>
<td>16</td>
<td>42.7 ~ 69.8</td>
<td>17.4 ~ 27.1</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Jun. 17</td>
<td>16</td>
<td>52.8 ~ 60.5</td>
<td>23.8 ~ 24.5</td>
<td>9</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul. 17</td>
<td>17</td>
<td>53.8 ~ 68.6</td>
<td>22.5 ~ 26.6</td>
<td>4</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug. 15</td>
<td>19</td>
<td>55.7 ~ 67.5</td>
<td>21.7 ~ 24.6</td>
<td>3</td>
<td>2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Sep. 14</td>
<td>21</td>
<td>56.5 ~ 67.0</td>
<td>22.0 ~ 23.4</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Oct. 12</td>
<td>20</td>
<td>60.0 ~ 68.5</td>
<td>23.7 ~ 24.5</td>
<td>6</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 17</td>
<td>19</td>
<td>61.8 ~ 66.7</td>
<td>24.2 ~ 24.4</td>
<td>10</td>
<td>9</td>
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<td></td>
</tr>
<tr>
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<td>14.3 ~ 24.6</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Feb. 16</td>
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<td>64.4 ~ 69.1</td>
<td>24.0 ~ 25.0</td>
<td>9</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Mar. 15</td>
<td>19</td>
<td>63.6 ~ 70.6</td>
<td>25.2 ~ 25.5</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Apr. 18</td>
<td>19</td>
<td>69.3 ~ 71.0</td>
<td>25.5 ~ 25.7</td>
<td>6</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>May 18</td>
<td>23</td>
<td>69.5 ~ 70.4</td>
<td>26.5 ~ 27.1</td>
<td>11</td>
<td>12</td>
<td></td>
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<tr>
<td>Jun. 14</td>
<td>17</td>
<td>61.4 ~ 69.5</td>
<td>22.9 ~ 25.8</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td></td>
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<tr>
<td>Jul. 27</td>
<td>17</td>
<td>64.9 ~ 69.2</td>
<td>22.7 ~ 23.4</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Aug. 26</td>
<td>21</td>
<td>42.5 ~ 63.9</td>
<td>15.6 ~ 22.6</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Sep. 20</td>
<td>18</td>
<td>45.3 ~ 67.8</td>
<td>15.7 ~ 24.1</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Note: M: male, F: female, I: indeterminate, H: hermaphrodites

mass, the sex can still be identified by the presence of the unresorbed gametes. Follicle walls are shrunken and broken into debris. (Figures 2e and 3e)

Stage 6: Indeterminate stage

Sex can not be identified and gonad is actionless after spawning; only connective tissues are observed in this degenerative state. (Figures 2f and 3f)

2. Hermaphrodities

Both sex gametes are present in follicles, spermatids surrounding free oocytes (Figure 3g), ovary developed earlier than testis are also identified. (Figure 3g-h).

3. Environment

The SST of sampling area in 2002 ranged from
26°C in March to 30°C in September (Figure 4). The salinity ranged from 31.2 psu at the lowest in August to 34.0 psu at the highest in November.

4. Development phases and spawning season

Gonadal development cycles of *Pinctada fucata* and *P. margaritifera* were determined by development phases of histological observations (Figures 2 and 3). Population spawning was characterized by a high frequency of releasing at Stage 4 (Figure 5a and 5c). In the year 2002, *P. fucata* had two spawning seasons, the major spawning occurred in May and the minor one in October. The gonadal development in the majority
Fig. 3. Microphotographs of gonadal development stages of female *P. margaritifera*. (a) early developing; (b) developing; (c) mature; (d) spawning; (e) spent; (f) indeterminate; (g and h) hermaphroditic.

onset in January (Figure 5a) and continued to April. In May, the gonads of most specimens were in Stages 3 and 4. After the main spawning period, the gonads exhibited a high proportion of Stage 5 and Stage 6 from June to August. In September, gonads began to develop again rapidly. About one month later, the second peak of maturity occurred in October and in November.

Gonadal development cycle in *Pinctada margaritifera* was similar to that in *P. fucata*, with two spawning seasons in a year (Figure 5c), though at different times. Individuals in stages 2 - 3 appeared in high frequency from March to June. The main spawning occurred in July, followed by August. After that time, gonads at Stages 1-2 were found in September. Oysters matured rapidly over a short time period during October, and the second spawning peak began in November.

5. Sex ratio

The monthly variation of sex ratio of *Pinctada fucata* and *P. margaritifera* are shown in Figure 5b and 5d respectively. In all *P. fucata* sampled, 135 (39.8%) specimens were males, 130 (38.3%) were females, 71 (20.9%) were indeterminate and 3 (0.9%) of *P. fucata* were hermaphroditic. Sex ratio (females: males) was 1:1.04, which was not significantly different from 1:1 ($\chi^2$ test, p > 0.05). In *P. margaritifera*, 162 (57.0%) specimens were males, 106 (37.2%) were females, 12 (4.1%) were indeterminate, and 4 (1.7%) were hermaphroditic. The sex ratio was 1:1.53 ($\chi^2$ test, p < 0.05). While males outnumbered the females in both species in the early spring of 2002, females tended to outnumber males during the spawning season, in May and in July. After spawning, the sex of oysters, with few exceptions, at stage 5 and 6 were indeterminable. In *P. margaritifera*, males outnumbered the females (around 70%) from March to June and in November of the year 2002, though, numbers of females increased from July to October. Hermaphroditic specimens of both *P. fucata* and *P. margaritifera* were observed around the months of spawning season.

**DISCUSSION**

Reproductive patterns and processes are quite varied in marine invertebrates [7]. Some species of potential culture or commercially shellfish are known to have annual reproductive cycles, but some bivalves have two spawning peaks per year, such as green mussel *Perna viridis* [21], pipi *Paphies australis* [9, 10], and hard clams *Mercenaria* spp. [8] and others. Similar characteristics have also been seen in pearl oysters, including *Pinctada albina* [33], *P. maxima* [18, 24, 29], *P. mazatlanica* and *Pteria sterna* [26].

The spawning peak of *P. fucata* varies by locality. *Pinctada fucata* spawns in June/July in Korea [3], in July in Iran [1], and in August/September in Japan [31, 45]. In addition, *Pinctada fucata* in Zamami Island, Okinawa, matures year round with a major peak in
winter and a minor one in summer [42]. This observation is different from the findings in the present study, even though the Okinawa is geographically close to Taiwan. The difference in spawning season of *P. fucata* between Zamami Island and Taiwan was probably a result of environmental conditions, including current, temperature, salinity and availability of food in these two areas.

In *Pinctada margaritifera*, the major spawning occurred in July and the minor one in November in Taiwan, which was similar to that in Kagoshima, Japan [27], but was different from that in the Red Sea, where spawning occurs in June [4]. Year round multispaing has been noted in French Polynesia [20], such behavior possibly being attributed to complicated factors, including latitudinal position [22, 24, 42] and temperature [5, 6, 14]. Mature individuals are induced and spawn when animals receive a thermal shock or are exposed in diluted sperm solution [9, 10].

Spawning season with the thermal-saline factors at the sampling site of the present observation (Figure 4) revealed that the first spawning peak of *P. fucata* in present study was associated with a rising of seawater temperature from 26.5°C in April to 28.5°C in May, and the second spawning occurred when water temperature decreased from 30°C in September to 28.3°C in October, which are significantly different (p < 0.05, ANOVA, the mean temperature and multiple comparison). Similarly, the first spawning of *P. margaritifera* occurred with the increase of temperature from 28.1°C in June to 29.2°C in July (p < 0.05). The second peak was also provoked by a rising of temperature from 28.3°C in October to 28.6°C in November (p < 0.05). This observation showed that the high temperatures in August and September seemed to have influence the release of gametes and the decrease in temperature made it possible for the gonads to quickly ripen in a short period, resulting in the second spawning.

In the first spawning seasons of both species, there was no obvious change in salinity (p > 0.05, ANOVA, the mean temperature and multiple comparison). Salinity, however, increased drastically from 31.6 psu in September to as high as 33.5 psu in October, and 34 psu in November corresponding to the second spawning periods for *P. fucata* and *P. margaritifera* (p < 0.05). Therefore, author suspect that the spawning seasons of *P. fucata* and *P. margaritifera* are associated with changes in water temperature, which are probably accompanied by fluctuations in salinity. In Japan, thermal stimulation has been used for a long time to promote gonad development and induce spawning in the seed production and pearl cultural technique used for *P. fucata* and *P. margaritifera* [30, 40, 43].

Hermaphroditic pearl oysters have been reported in the past [24, 35-37, 39]. Wada [44] and Tranter [35-37] both suggested that pearl oysters, such as *P. fucata*, *P. margaritifera*, *P. maxima* and *P. albina* were protandric hermaphrodites. Sex changes from male to female, and female to male were also described. The present study found the protandric trend condition for *P. fucata* and *P. margaritifera* in Taiwan. A few hermaphrodite individuals were also detected at the time close to spawning seasons. The predominance of males was usual in early development stages in both *P. fucata* and *P. margaritifera*, but females outnumbered males during the spawning season. *Pinctada fucata* and *P. margaritifera* had a protandric tendency. While this study only found a few hermaphrodites they are often found in species of genera *Pinctada*. The prototypic tendency of *P. fucata* was also reported by Tateishi and Adachi [31] Japan. Uemoto [39] found that the sex ratio in *P. martensii (= fucata) was 1 ♀ : 1.29 ♂ (43.7% ♀ : 56.3% ♂) in the three and four-year-old adult individuals. In other bivalves, the females become predominant with increase of size and age, as documented in *Arctica islandica* [23] and in *Crassostrea virginica* [15]. However, there was no significant difference in nber between males and females for oysters above 40 mm in shell height in this study.

In addition, sexuality indeterminate individuals appeared in the period after spawning, especially in summer. This phenomenon was possibly due to the gonad entering a resting period after spawning, the gametes cell starting to degenerate.

In conclusion, although temperature has been the important environmental factor in bivalve reproductive physiology [25], salinity is also an element that needs to be taken into consideration. This study confirmed that reproductive cycle of *Pinctada fucata* and *P. margaritifera* in Taiwan waters has two spawning peaks but with different cycles in different months a year. Temperature may play an important role in oyster maturity and spawning.

ACKNOWLEDGEMENTS

This study was funded by the Fisheries Agency, Council of Agriculture, Executive Yuan (92AS-2.5.1-FA-F1.11-3). Sincere gratitude to Professor Aoki and associate professor Yamakawa of the Tokyo University for their valuable comments on an earlier draft, and associate professor Yoshinaga for guidance in microphotographic technics. I would like to express my special thanks to Mr. C. Y. Liu for his help in measurement and histological sectioning for this study. I would further like to extend thanks to Dr. K. M. Liu National Taiwan Ocean University and Dr. T. Okutani for reviewing this manuscript.
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