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Seasonal Changes of Reproductive Activity and Biochemical Composition of Pen Shell *Atrina pectinata* Linnaeus, 1767 in Bohai Sea, China

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Abstract Seasonal variations in the biochemical composition and the reproductive cycle of pen shell *Atrina pectinata* in Bohai Sea were investigated from May 2013 to April 2014. Histological analysis indicated that the reproductive cycle of *A. pectinata* can be divided into two phases, inactive stage and gametogenesis, which were equally and significantly influenced by seawater temperature and food availability. Gametogenesis began in late autumn (October), and completed in June and July. Spawning took place in August, coinciding with the highest water temperature and the richest phytoplankton. The significantly high glycogen content in adductor muscles sustained throughout the late active and ripeness stages, but plummeted during spawning and inactive stages. The protein content in female gonads exhibited a synchronous increase along with oocyte diameter and lipid content, suggesting that the female gonads could accumulate protein and lipid for vitelline in *A. pectinata*. Furthermore, the RNA/DNA ratio was found to be a useful index to indicate the level of gonad maturation in both males and females. The findings of the present study provided a foundation for the fishery resource administration and the aquaculture development of this species.

Key words biochemical composition; reproductive cycle; environment factor; *Atrina pectinata*; seasonal variation

1 Introduction

Pen shell *Atrina pectinata* is a commercially important bivalve belonging to family Pinnidae. It widely distributes in silty sand or muddy sediments at a water depth up to 20–50 m along the coasts of China, Korea and Japan (Min, 2004; Okutani, 2000; Qiu *et al*., 2014). The large adductor muscle, which is regarded as a delicacy, has long been a favorite in East Asia (Lee *et al*., 2015). The annual production of *A. pectinata* in China declined from about 40183 tons in 2003 to 17618 tons in 2014 (CFY, 2005, 2015). This not only posed a threat to the fishery sustainability of this species, but also compromised its productive potential and the local ecological balance. With the over-exploitation of natural stocks and growing market demand, it has been a significant species for aquaculture. However, after decades of research, there is no breakthrough of the artificial breeding of pen shells owing to the bottleneck in artificial promotion of maturity and spawns. According to previous researches, brood stock condition could serve to solve this problem. Therefore, understanding the natural reproductive cycle of *A. pectinata* is essential for establishing successful artificial breeding production.

In bivalve mollusks, the reproductive cycle is closely related to energy storage and utilization, which is affected by environmental conditions such as temperature and food availability (Giese, 1969; Joaquim *et al*., 2008; Berthelin *et al*., 2000). The growth and gametogenesis of bivalves are promoted or delayed under different temperatures and food conditions (Utting, 1993; Utting and Millican, 1997; Kang *et al*., 2000; Fearman and Moltschaniwskyj, 2010). Gametogenesis is an energy-demanding process that requires the mobilization of nutrients from ingested food or the reserves from body tissues (Ruiz *et al*., 1992). Reserves in forms of protein substrates, lipid, and glycogen are synthesized when food is abundant and utilized in the production of gametes due to sizeable metabolic demand (Acarli *et al*., 2015). Different species or different populations of the same species vary in their significant substrates and how the timing of their consumption is related to gametogenesis (Barber and Blake, 1981). In general, there are two types of animals in terms of mode of energy storage and gametogenesis (Heard and Guckert, 1970; Bayne, 1976; Matias *et al*., 2013). For conservative pattern bivalves such as *Cyclina sinensis* (Yan *et al*., 2010) and *Fulvia mutica* (Liu *et al*., 2008), energy used for gametogenesis arises from the substrates stored in various organs; while for opportunistic pattern bivalves, it comes from recently ingested en-

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ergy from abundant food intake (Bayne, 1976). The reproductive strategy could provide detailed knowledge on the effects of local environmental conditions on its storage metabolism in terms of the reproductive cycle (Dridi *et al*., 2007).

In the present study, we have investigated an annual reproductive cycle and seasonal change in the biochemistry of different tissues of *A. pectinata* from Bohai Sea. To address the energy flow and what contributes to the gametogenesis in different organs, the major constituents (viz. glycogen, lipid, protein) from four main parts (viz. adductor muscle, mantle, gill, and gonad-visceral mass) of *A. pectinata* examined. This information is useful in the future for both the aquaculture production of pen shell and the programs of enhancing and restoring natural stocks in Bohai Sea.

2 Materials and Methods

2.1 Origin of Material

About 30–35 specimens were collected by scuba diving each month from May 2013 to April 2014 from Bohai Sea (38°21′–38°33′N; 120°64′–120°71′E), China (Fig.1). After being transported to the laboratory in an icebox, they were dissected carefully using stainless steel knife to obtain adductor muscle, mantle, gill, and gonad-visceral mass including the digestive system, nerve ganglia, and kidney-pericardium region for the analysis of biochemical composition. Because of the difficulty in separating the organs, the gonad-visceral mass was analyzed as a unit. A part of the gonad-visceral tissue was excised from each specimen for histology examination. The separated tissues were frozen and then stored at −80℃ until analysis.

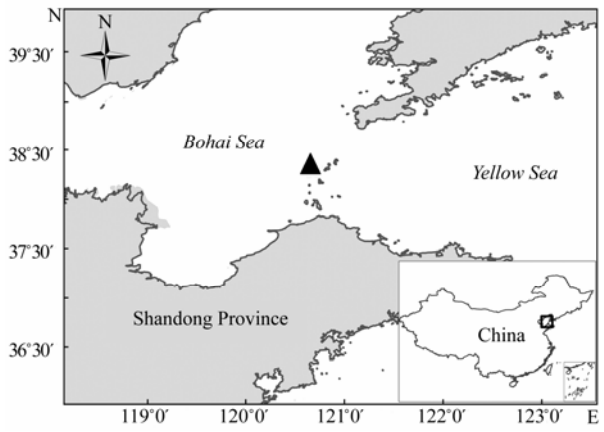

Fig.1 Map showing the sampling site (▲).

The temperature and salinity of the surface seawater were measured *in situ* during sampling with a mercury bulb thermometer and portable refractometer. Water (about 3 L) were collected at a depth of approximate one meter below surface at sampling site. The concentration of chlorophyll *a* was determined according to Parsons (1984).

2.2 Condition Index

The condition index (*CI*) of the bivalve was calculated following the formula below (Walne, 1976). *CI* = (Dry weight of the soft tissues/dry shell weight) × 100. Five individuals each monthly specimen were used in *CI* analysis.

2.3 Histology

For histology, the 5-mm cross section of the gonad visceral tissue of each specimen was placed in Bouin solution (saturated picric acid:formalin:glacial acetic acid = 15:5:1) at 4℃ for 24 h. After fixation, they were transferred to 70% ethanol for storage. Posteriorly, sections were dehydrated in a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. The sections, 5-μm in thickness, were cut, mounted on glass slides and stained with hematoxylin and eosin (H&E). Each histological section was examined under microscopy to determine the gametogenesis stages. Five stages of gonadal development were identified according to Ivell (1979). When more than one developmental stages occurred simultaneously within a single individual, the assignment of the stage criteria decision was based upon the condition of the majority of the section. The diameter of 500 oocytes cut through the nucleus from 10 individuals was measured to trace the annual gametogenesis of pen shell.

2.4 Biochemical Analysis

For the biochemical components characterization, the quantity of glycogen, lipid, protein, and nucleic acids were estimated. Each determination of biochemical compounds was carried out in triplicate on pooled tissues of 12–15 individuals. The glycogen content was analyzed with a minor modifications of the anthrone, sulfuric acid method described by Horikoshi (1958). Fifty mg powdered, freeze-dried materials separated with different tissues and sexes were, respectively, suspended in 60 volumes of 30% KOH. They were saponified by keeping at 100℃ for 30 min. After cooling, a portion of the saponified mixture was treated with 5 mL cold 0.2% anthrone-sulfuric acid solution for 10 min, the absorbance of the resulting colored complex was measured at the wavelength of 620 nm. The lipid concentration was analyzed using the gravimetric method. Total lipids were extracted from 200 mg of dried, powdered samples in petroleum ether by using an automatic Foss extraction system (Soxtec 2050; FOSS, Sweden). Soluble protein levels in tissues were determined according to the method of Marion (1976), using bovine serum albumin as the reference. Fresh tissue (100 mg) was homogenized in 2 mL saline solution. After being centrifuged and diluted, the crude extract sample was mixed with Brilliant Blue G; then the absorbance value was measured at 595 nm. After the tissues had been homogenized in 20 volumes of distilled water, 1 mL of each homogenate was used for determining nucleic acid (DNA and RNA) content according to the modified Schmidt-Thammhauser-Schnerder method (Nakano, 1988). Nucleic acids were precipitated with ethanol and washed with a mixture of ethanol and ether. RNA was separated by alkaline hydrolysis, and DNA was

hydrolyzed with perchloric acid. DNA and RNA contents were determined by measuring the absorbance at 260 nm.

2.5 Statistics

All data were tested for homoscedasticity and normality before running statistical test. One-way ANOVA was used to assess significant variations in the condition index and oocyte diameter among different months followed by Duncan's test (α=0.05). Two-factor ANOVAs were employed to compare the biochemical parameter on each separate tissue using sex and month as the factors, followed by post-hoc comparisons using Duncan's test (α=0.05). When the values of each biochemical parameter were significantly different between sexes, the values of each biochemical composition of each separate tissue were compared in each sex among months using one-way ANOVA followed by post-hoc comparisons using Duncan's test (α=0.05). Pearson's correlation analysis was applied to describe the relationship between studied parameters. The software SPSS 16.0 was used for data analyses.

3 Results

3.1 Environmental Parameters

Monthly variations in seawater temperature, salinity, and chlorophyll *a* concentration at the sampling site are shown in Fig.2. Seawater temperature ranged from 2.9℃ to 25.2℃ with a maximum in August and a minimum in February. Mean salinity ranged from 32.6 (December 2013) to 29 (August 2013). Seasonal variations in chlorophyll *a* concentration were similar to seawater temperature. It reached a maximum of 12.5 μg L^{-1} in August and a minimum of 1.5 μg L^{-1} in December. The chlorophyll *a* concentration was significantly related to the seawater temperature (Table 1) (P<0.05).

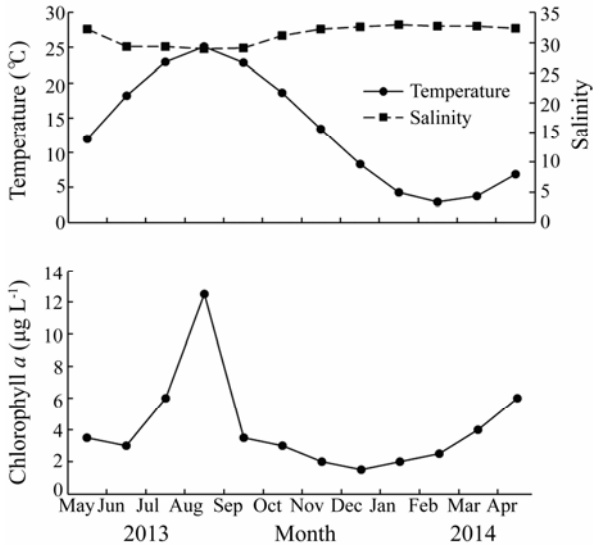

Fig.2 Monthly variations in seawater temperature, salinity and chlorophyll *a* concentration at sampling site.

Table 1 Matrix of Pearson correlation coefficients of the environmental parameters and the biological parameters

Parameter	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
T (A)	0.68*	0.75*	0.79*	−0.95**	−0.46	0.32	0.52*	0.63*	0.27	0.70*	0.56*	0.10	−0.19	0.78*	0.70*	0.52*
Chl *a* (B)		0.39	0.50*	−0.66*	−0.16	0.06	0.45	0.23	0.28	0.50*	0.33	0.53*	0.02	0.72*	−0.21	0.44
CI (C)			0.99**	−0.83**	−0.47	0.60*	0.75*	0.84**	0.61*	0.60*	0.78*	0.16	−0.14	0.75*	−0.89**	0.32
OD (D)				−0.84**	−0.38	0.63*	0.81**	0.83**	0.62*	0.57*	0.76*	0.22	−0.11	0.77*	−0.83**	0.33
S (E)					−0.63*	−0.25	−0.51*	−0.65*	−0.33	−0.77*	−0.68*	−0.20	0.16	−0.87**	0.81**	−0.5*
GVMgly (F)						0.03	0.08	−0.17	−0.08	−0.49	−0.34	−0.14	0.43	−0.46	0.63*	−0.26
AMgly ♀ (G)							0.88**	0.55*	0.67*	0.04	0.50*	0.17	0.08	0.20	−0.43	0.04
AMgly ♂ (H)								0.64*	0.78*	0.26	0.61*	0.39	0.13	0.54*	−0.52*	0.15
GVMpro ♀ (I)									0.45	0.57*	0.66*	0.07	0.07	0.59*	−0.71*	0.24
GVMpro ♂ (J)										0.26	0.46	0.27	0.04	0.42	−0.41	0.04
AMpro (K)											0.64*	0.26	0.02	0.82**	−0.54*	0.63*
GVMlip ♀ (L)												0.51*	0.15	0.70*	−0.73*	0.36
GVMlip ♂ (M)													0.36	0.45	0.00	0.28
AMlip (N)														0.08	0.26	0.06
GVMnu ♀ (O)															−0.59*	0.62*
GVMnu ♂ (P)																−0.17

Notes: A, temperature; B, chlorophyll *a*; C, condition index; D, oocyte diameter; E, salinity; F, gonad-visceral mass glycogen; G, adductor muscle glycogen of female; H, adductor muscle glycogen of male; I, gonad-visceral mass protein of female; J, gonad-visceral mass protein of male; K, adductor muscle protein; L, gonad-visceral mass lipid of female; M, gonad-visceral mass lipid of male; N, adductor muscle lipid; O, gonad-visceral mass RNA/DNA ratio of female; P, gonad-visceral mass RNA/DNA ratio of male; Q, adductor muscle RNA/DNA ratio (AMnu) of *A. pectinata*. * and ** are significant at 0.01 < P < 0.05 and $P \leq 0.01$, respectively.

3.2 Condition Index and Oocyte Diameter

A significant difference was observed in the annual *CI* of *A. pectinata*, which varied between 24.9% and 10.3% (Fig.3). The maximum level was recorded in July and then declined sharply to the minimum in September. In the late spring and early summer, the value increased rapidly to the maximum in July. The *CI* showed a significant correlation with oocyte diameter (Table 1) (P < 0.01). Seasonal variation in the mean oocyte diameter was also clear and displayed a similar pattern with that observed from the *CI* (Fig.4). Both *CI* and oocyte diameter increased from May to July, indicating that the gametogenesis entered the rapid growth phase. The *CI* and oo-

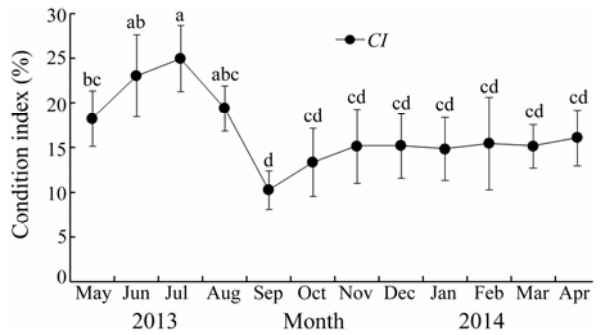

Fig.3 Monthly variation in the *CI* of *Atrina pectinata*. Means not sharing the same superscript are significantly different.

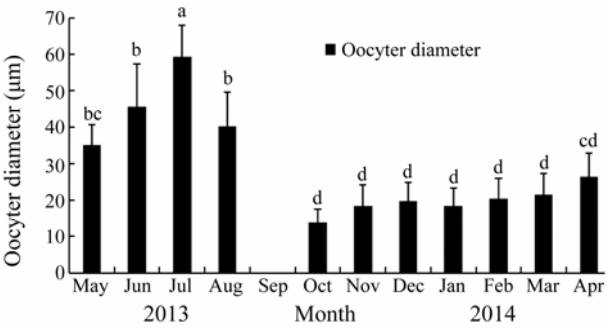

Fig.4 Monthly variation in the oocyte diameter of *A. pectinata*. Means not sharing the same superscript are significantly different.

cyte diameter peaked in July, and then declined sharply in August. Such sudden decrease of *CI* and oocyte diameter in August coincided with the spawning.

The histological analysis of ovarian and testicular tissues during reproductive cycle are shown in Figs.5 and 6. At stage 0, the gametes had almost disappeared and could not be recognized. During stage I, the follicles with small oogonia or spermatogonia were poorly developed in a few individuals and surrounded by large connective tissues, indicating that the gametogenesis just began. Stage II represented a period of moderate gonadal development. At this stage, the follicles began to distend, connective tissue nearly disappeared. During stage III, gametogenesis completed, although spawning had not occurred. The follicles appeared to occupy most of the gonadal region and to be full-grown mature gametes (Fig.5, III). The diameter of oocyte was much bigger than before. During stage IV, the gonads were partly used up. The follicles were shrunk, and a few gametes were remained.

The percentage distribution of different gametogenesis stages in female and male gonads observed during the period of study is shown in Fig.7. This species presents a synchronous seasonal pattern of gonadal development in both sexes. Gonad gametogenesis started in October. Most individuals were in the stage II during May and ripened during July. The onset of spawning occurred in August, and nearly 90% of them had spawned in this month. In September, all the specimens were in stage 0.

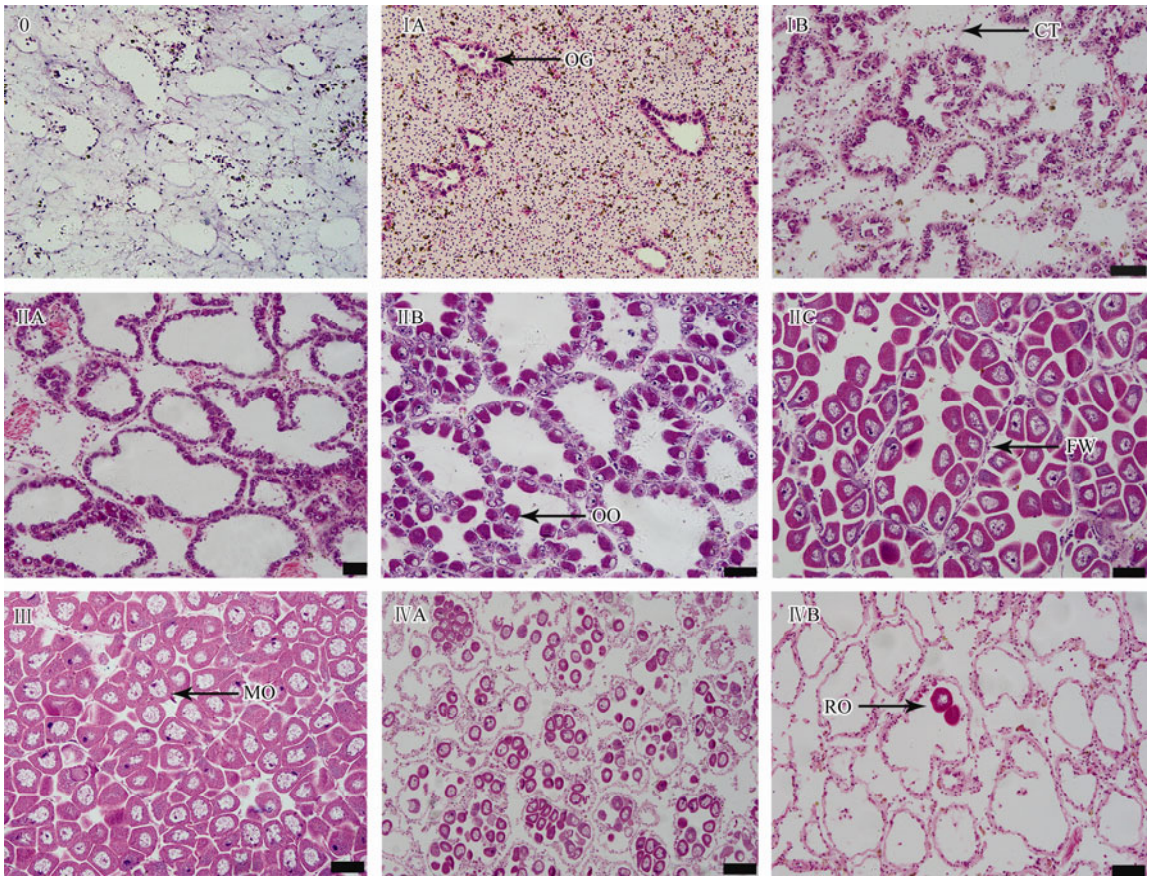

Fig.5 Photomicrographs of the histological gonadal stages of female *A. pectinata*. OG, oogonia; OO, oocyte; MO, mature oocyte; RO, residual oocyte; FW, follicle wall; CT, connective tissue; 0, Inactive; I, Early active; II, Late active; III, Ripe; IV, Spent; Scale bar: 50 μm. The letters mean different develpments in the same stage.

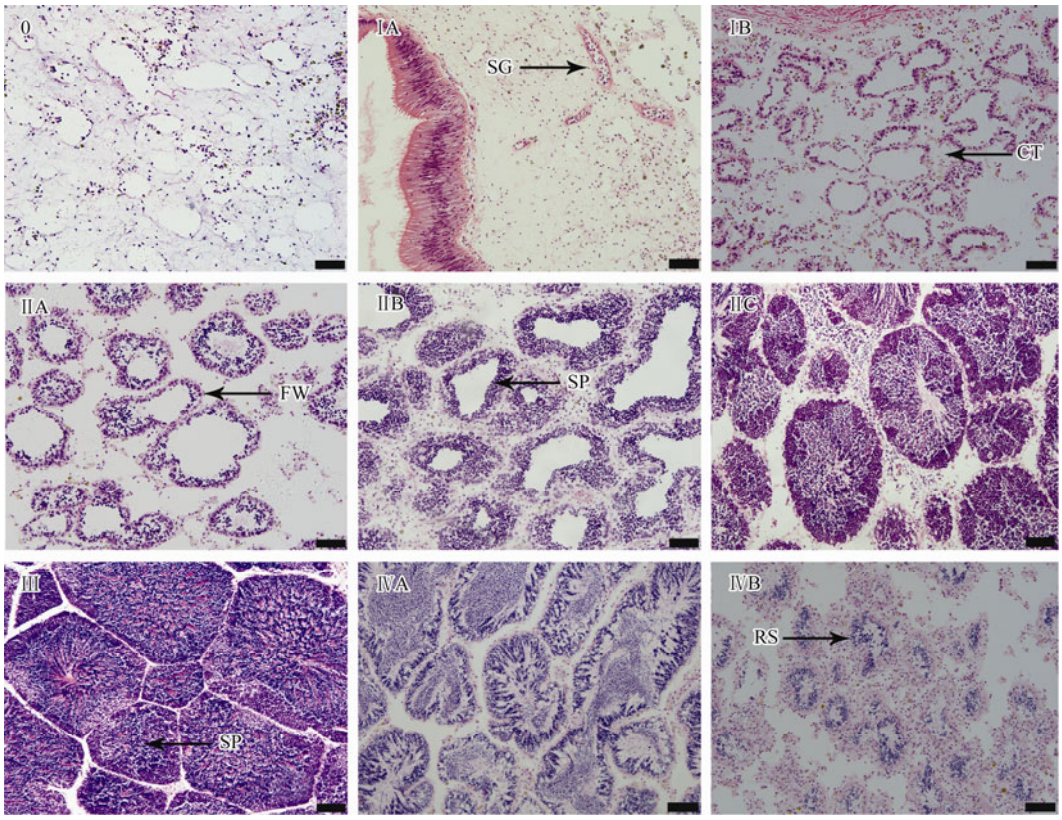

Fig.6 Photomicrographs of the histological gonadal stages of male *A. pectinata*. SG, spermatogonia; SP, spermatozoa; RS, residual spermatozoa; FW, follicle wall; 0, Inactive; I, Early active; II, Late active; III, Ripe; IV, Spent; Scale bar: 50 μm. The letters mean different developments in the same stage.

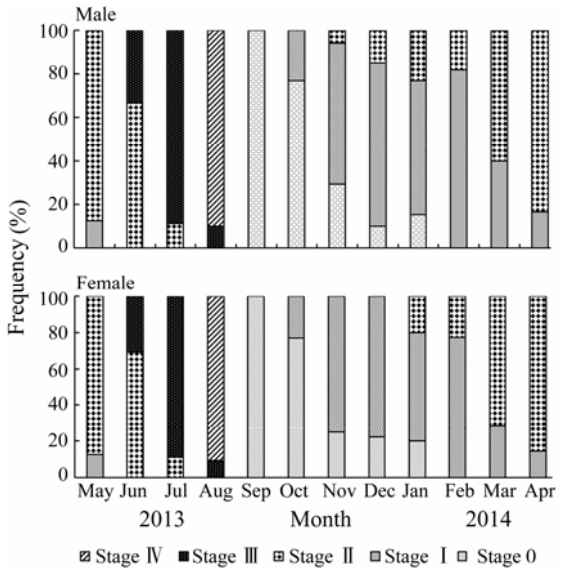

Fig.7 Seasonal distribution of *A. pectinata* at different stages of gonadal development.

3.3 Biochemical Composition

Seasonal variations of the contents of glycogen, lipid, protein, and RNA/DNA ratio in different body parts (gonad-visceral mass, adductor muscle, mantle, and gill) from both sexes are shown in Figs.8–11.

The glycogen content in adductor muscle had a similar pattern in both females and males (Fig.8). In adductor muscle, the glycogen content decreased gradually from July (28.2% in female and 32.7% in male) to a minimum level of 2.9% in September, and then recovered in October. The content of glycogen in gonad-visceral mass below 10.5 mg g^{-1} throughout the year showed a similar pattern with Yurimoto (2015), and there was no significant correlation with the glycogen content in adductor muscle (Table 1). The minimum level in the gonad-visceral mass (1.8% of females and 2.4% in males) was observed in June, showing the different tendency with the adductor muscle. The glycogen content in mantle varied from 2.4% to 7.2%, and also showed relatively lower values during the inactive stage. The glycogen content in gill was less than 2.5% throughout the year. The results revealed that the adductor muscle in *A. pectinata* was the main storage tissue for glycogen (Lee *et al.*, 2015). Two-factor ANOVA analysis indicated that there were no significant differences between sexes in the glycogen content of the gonad-visceral mass, mantle, and gill ($P>0.05$), though the glycogen content changed significantly over the year ($P<0.05$). The glycogen content in the adductor muscles showed significant monthly variations, as well as significant differences between sexes ($P<0.05$).

The lipid content in the female and male gonad-visceral mass showed a seasonal pattern with the minimum value in September (Fig.9). The lipid in female gonad-visceral mass reached the highest level in July followed by an apparent decrease in August; however, the male gonad-visceral mass reached a high level in August followed by an apparent decrease in September. The lipid

content in adductor muscle showed a similar level through the year with an except during November and January. The lipid content in the gonad-visceral mass and gill showed significant monthly variations, as well as significant differences between sexes ($P<0.05$).There was no significant negative correlation between the lipid content in the adductor muscle and the gonad-visceral mass (Table 1).

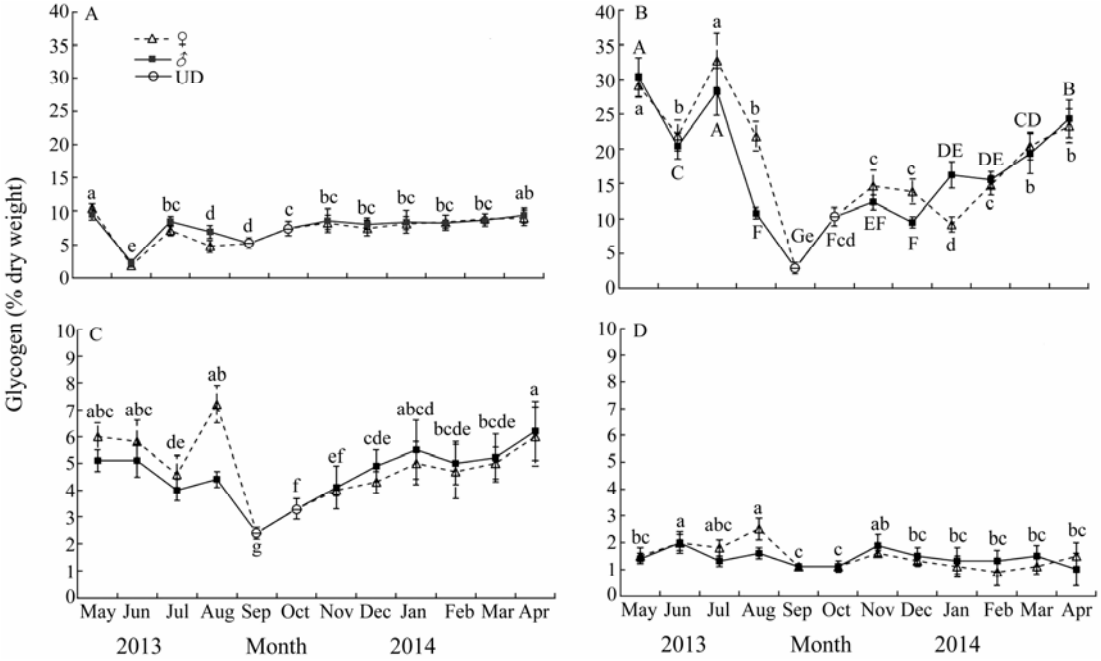

Fig.8 Monthly variation of the glycogen content (% dry weight) in *A. pectinata* throughout the year (Mean±SE, $n=3$). Different letters indicate significant monthly difference; different capital and lowercase letters indicate significant monthly difference between male and female ($P<0.05$). A, gonad-visceral mass; B, adductor muscle; C, mantle; D, gill. ♀, female; ♂, male; UD, undistinguished.

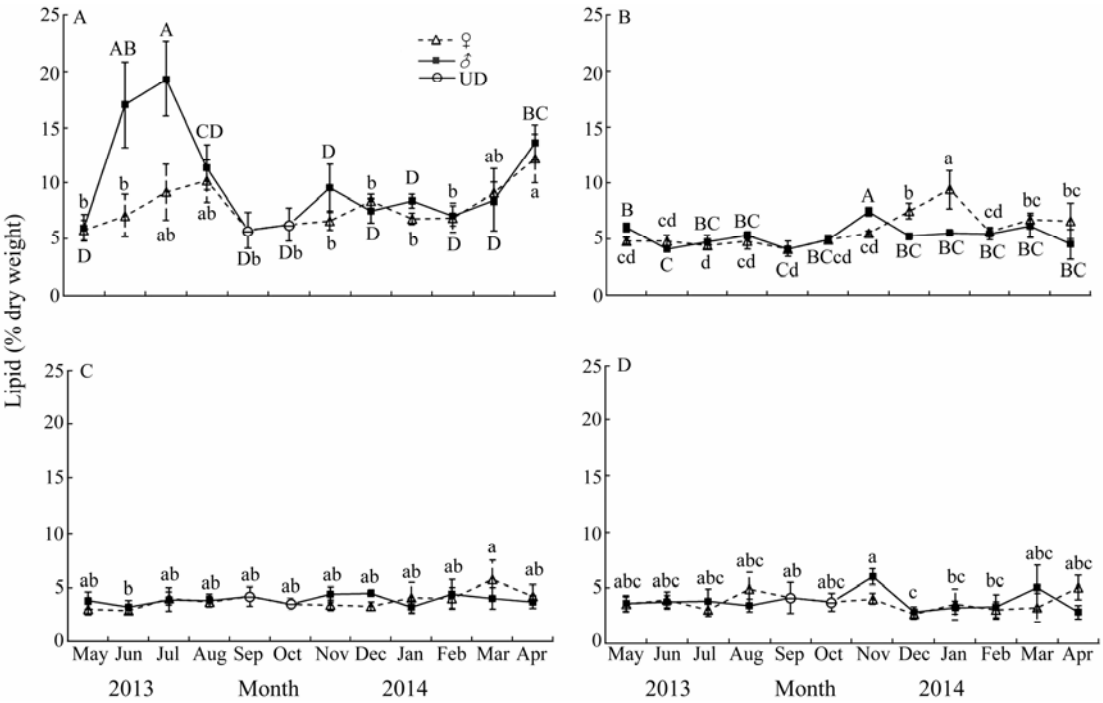

Fig.9 Monthly variation of the lipid content (% dry weight) in *A. pectinata* throughout the year (Mean±SE, $n=3$). Different letters indicate significant monthly difference; different capital and lowercase letters indicate significant monthly difference between male and female ($P<0.05$). The meaning of the symbol was Symbolic representation is the same as Fig.8.

The protein content in the studied tissues had an increasing tendency after the spawning period and dropped sharply from August to September (Fig.10). In male gonad-visceral mass, the protein content increased from September and reached the maximum level in May. However, the female gonad-visceral mass had a converse ten-

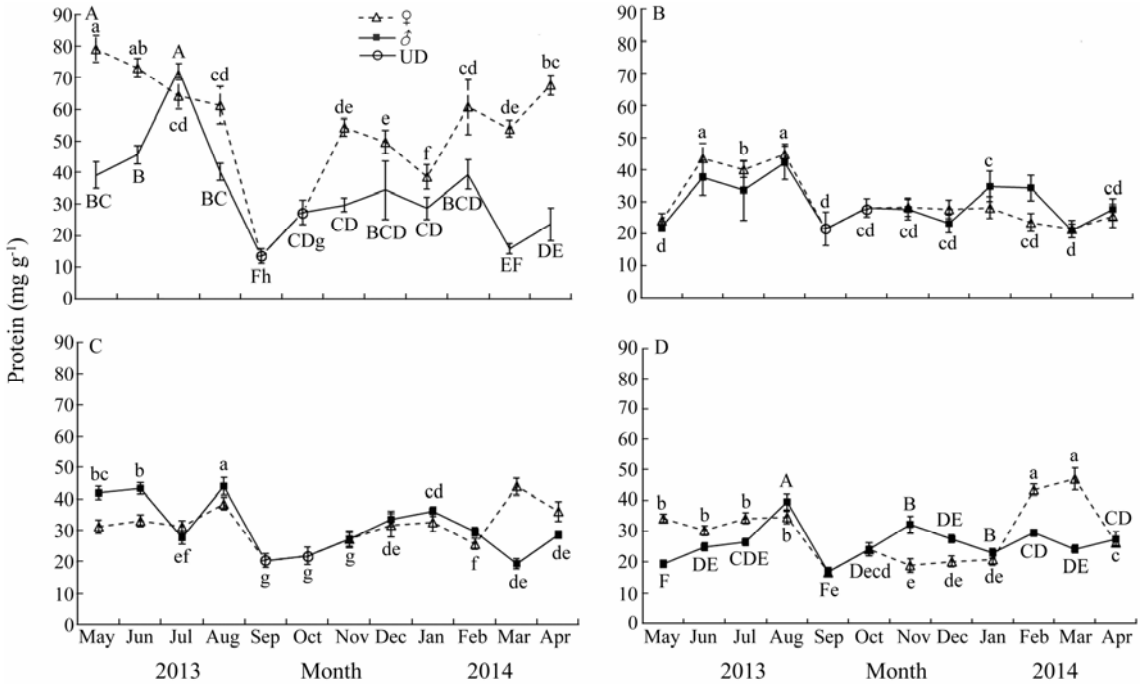

Fig.10 Monthly variation of the protein content (‰ wet weight) in *A. pectinata* throughout the year (Mean±SE, $n=3$). Different letters indicate significant monthly difference; different capital and lowercase letters indicate significant monthly difference between male and female ($P<0.05$). The meaning of the symbol was Symbolic representation is the same as Fig.8.

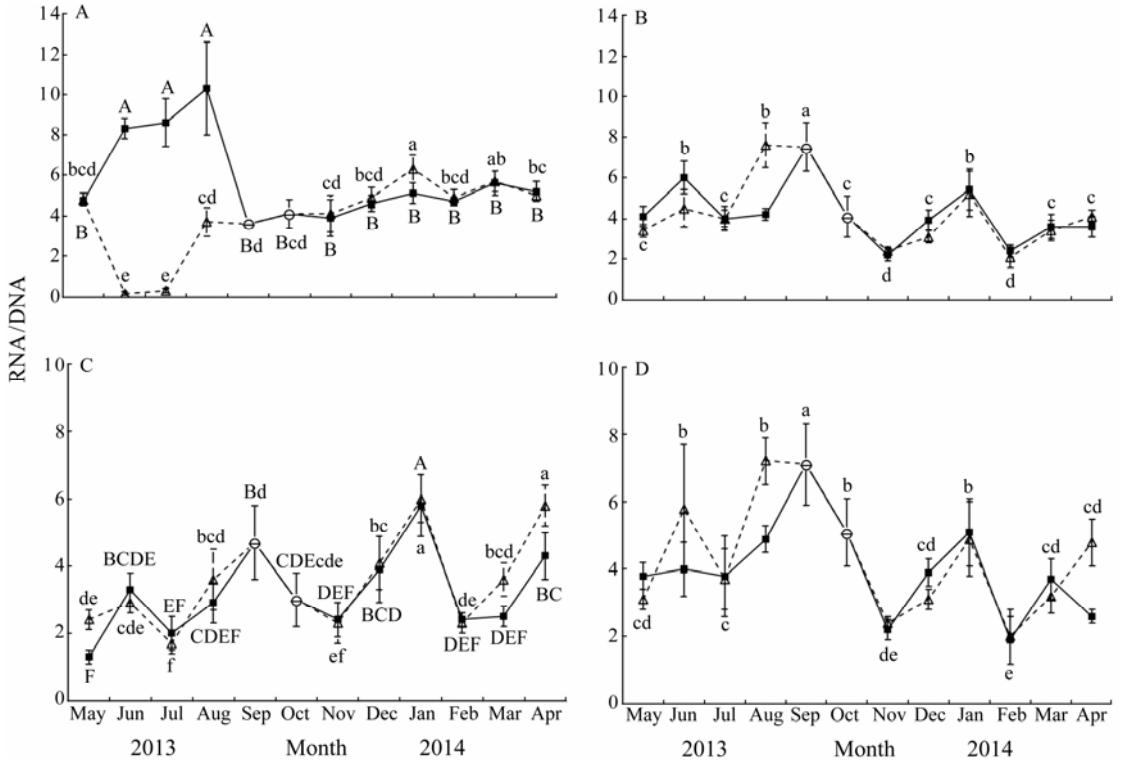

Fig.11 Monthly variation of the RNA/DNA ratio in *A. pectinata* throughout the year (Mean±SE, $n=3$). Different letters indicate significant monthly difference; different capital and lowercase letters indicate significant monthly difference between male and female ($P<0.05$). The meaning of the symbol was Symbolic representation is the same as Fig.8.

dency during May to July. The protein content in adductor muscle stayed at a high level during June to August and decreased sharply to the minimum in September ($21.4\,mg\,g^{-1}$). There was no significantly negative correlation with the glycogen and lipid in adductor muscle and gonad-visceral mass (Table 1), which mean that there was no clear conversion between glycogen, lipid, and protein in adductor muscle and gonad-visceral mass. The protein content in the gonad-visceral mass and gill showed significant monthly variations, as well as significant differ-

ences between sexes ($P<0.05$).

The RNA/DNA ratio in the female gonad-visceral was negatively correlated with the ratio in male (Table 1) ($P<0.05$). It was increased slightly from September to May and then increased significantly in summer (June to August). However, it showed a similar tendency from September to May in male but followed with a significant decrease from 4.8 in May to 0.3 in July. The RNA/DNA ratio in the adductor muscle and the gills showed a similar tendency, with the lowest level of value in December and February. The ratio in mantle showed a high level of value in January and March (Fig.11). Two-factor ANOVA analysis indicated that there were no significant differences between sexes in RNA/DNA ratio of the adductor muscle and gill, though it changed significantly over the year ($P<0.05$). The ratio of the gonad-visceral mass and mantle showed significant monthly variations, as well as significant differences between sexes ($P<0.05$).

4 Discussion

4.1 Gametogenesis and Environmental Factors

By affecting the availability of food or metabolic rate, water temperature is considered to be the principal environmental parameter of gametogenesis of marine bivalves (Park *et al*., 2001; Viña, 2002). In terms of annual reproductive cycle of marine bivalves, different species and different populations of the same species vary in the number of spawning events. The time and duration of spawning periods of bivalves are directly influenced by the geographical latitude which determines the temperature of seawater (Chávez-Villalba *et al*., 2002; Da Silva *et al*., 2009; Park *et al*., 2011). Previous studies demonstrated that *A. pectinata* had two spawning pulses (May-November) in East China Sea (Wang *et al*., 2000) and South China Sea (Luo *et al*., 1990), and one spawning event (June-August) in Yellow Sea (Qiu *et al*., 2014). In the present study, the reproductive cycle of *A. pectinata* in Bohai Sea was characterized by a unimodal cycle with a resting stage (September), a gametogenesis stage beginning in late autumn, and a spawning event in summer (August). As for *A. pectinata*, there are usually two spawning events in populations in tropical and subtropical regions. By contrast, there is only one spawning event in temperate regions. Although there exists no clear specific correlation between temperature and gonadal maturation, previous studies showed that gametogenesis was affected by both temperature and the exposure period (Mann, 1979).

Food availability is another important factor influencing the gametogenesis of bivalves (Baghurst and Mitchell, 2002; Frias and Segovia, 2010; Fournier *et al*., 2012). Chlorophyll *a* was employed as a conventional tool to compare the food availability (Li *et al*., 2009). In the current study, chlorophyll *a* exhibited a significantly positive relationship with oocyte diameter (Table 1). Food availability could provide enough energy for the gonadal development of *A. pectinata*. It was observed that a high food availability would facilitate the gametogenesis of *A. pectinata* in Korea (Lee *et al*., 2015), and similar results were reported in *Mytilus edulis* (Newell *et al*., 1982) and *Crassostrea gigas* (Kang *et al*., 2000). In addition, spawning duration time of *A. pectinata* coincided with the highest level of chlorophyll *a*, ensuring sufficient food supply in the water for the released larvae. Such relationship between food availability and spawning duration time was also found in *Mactra chinensis* (Li *et al*., 2011), *F. mutica* (Liu *et al*., 2008) and *C. sinensis* (Yan *et al*., 2010).

4.2 Biochemical Content and Reproductive Strategy

In bivalve mollusks, the energy storage and utilization are related closely to the reproductive cycle (Giese, 1969; Berthelin *et al*., 2000; Joaquim *et al*., 2008). The biochemical composition affected by environmental factors as well as interspecific-intraspecific differences are the reflection of those metabolic activities (Newell and Bayne, 1980; Gabbott, 1983; Mackie and Ansell, 1993).

Glycogen, the main energy reserve in adult bivalves, can be assimilated into an energy source for growth as well as stored in specific tissue as energy reserve during gametogenesis (Mathieu and Lubet, 1993; Robert *et al*., 1993; Liu *et al*., 2008). The results of the present study indicated that the glycogen content increased during the gametogenesis, and plunged in the spawning period. The largest seasonal fluctuation during the spawning stage indicated that reserved glycogen in developing stages could be used as an immediately available energy source for spawning (Racotta *et al*., 1998; Ruiz-Verdugo *et al*., 2001; Racotta *et al*., 2003). At the same time, a decline was identified in the level of glycogen in adductor muscles and gonad-visceral mass, accompanied with the decline of chlorophyll *a* in June, which indicates that glycogen could support the synthesis of biochemical substances when the food supply is in shortage.

Protein is a major organic component of mature oocytes, and also serves as an energy reserve in adult bivalves (Utting and Doyou, 1992; Galap *et al*., 1997). In the present study, the protein content in gonad-visceral mass exhibited a significant difference between males and females during maturation stage. The increase in female gonad-visceral mass indicated that the protein was accumulated in the vitelline of oocytes during the maturation stage (Barber and Blake, 1981; Dridi *et al*., 2007). The same pattern of protein accumulation in gonad has also been observed in *C. sinensis* (Yan *et al*., 2010) and *C. gigas* (Li *et al*., 2000). Conversely, the decrease of protein content in male gonad-visceral mass indicated that the protein could also be an energy resource for *A. pectinata*. Moreover, similar pattern was also observed in adductor muscles, gill and mantle. In addition to gonad-visceral mass, protein content soared to its highest level in August, followed by a decrease in September. However, the glycogen in adductor muscle was almost depleted in September. The dramatic decrease of protein indicated that the protein could be used as an energy source after glycogen reserves were exhausted.

Lipid content has been considered as an indicator of gamete quality (Pazos *et al*., 1997). In the present study, the lipid content in gonad-visceral mass exhibited a seasonal pattern associated with the gametogenesis. It increased prior to spawning and then plummeted, and the similar pattern was reported in *M. chinensis* (Li *et al*., 2011). The rapid increase of lipid in female gonad-visceral mass was identified in near-ripe or ripe stage (June-July), indicating that lipid was accumulated in ripening eggs (Yan *et al*., 2010). As males mostly accumulate membrane lipid such as sterols or phospholipids which are not energetic reserves (Soudant *et al*., 1996), the lipid content in male gonad-visceral mass was significantly lower than that in female from June to July. The lipid content in adductor muscles did not present a seasonal pattern during the reproductive cycle and maintained a low level, indicating lipid is not an important energy source in adductor muscle (Lee *et al*., 2015; Yurimoto, 2015).

Since RNA participates in the protein synthesis, the RNA/DNA ratio has been used as an index of the synthetic activity of protein in many species as the DNA content is identical per somatic cell of a given species (Nakata *et al*., 1994; Okumura *et al*., 2002). In the present study, the RNA/DNA ratio represented a similar pattern throughout the year in addition to the gonad-visceral mass. Furthermore, the changes of the RNA/DNA ratio in female gonad-visceral mass synchronized with the oocyte diameter, and reached the highest level in August. During the process, the protein and lipid as the constituent of oocyte showed a same tendency. Interestingly, glycogen content showed a negative relation with RNA/DNA ratio in female gonad-visceral mass, because it was a major energy sources in biosynthesis. It shows that the RNA/DNA ratio in female gonad-visceral mass has a link with gametogenesis. Conversely, as the number of sperms increases during the ripe stage, the RNA/DNA ratio in male gonad-visceral mass exhibited a significant decrease to the lowest level from June to July. This significant difference between males and females indicated that the RNA/DNA ratio could be used as an indicator of maturation for both males and females.

In conclusion, this study is the first effort to describe the reproductive strategy of *A. pectinata* from Bohai Sea in relation to its nutrient storage and consumption of four different tissues in both sexes. Since the nutrient content of main storage organs tended to increase throughout gamogenesis before spawning and there was no clear conversion between glycogen, lipid and protein, this bivalve could be characterized as an opportunistic species. The pen shells in Bohai Sea are characterized by a single spawning period in August with the highest seawater temperature and chlorophyll *a* levels due to the monocyclic gametogenesis throughout the year. Gametogenesis took place in late autumn and winter depending on the energy absorbed from ingested food. To sum up, the data obtained in this study show the biochemical components and the reproductive strategies of *A. pectinata* populations in Bohai Sea, providing a basis for the management of fishery resource of this species and the initiation of its aquaculture.

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