J. Fish. Sci. Tech. 4(1), 18~24, 2001

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Seasonal Variations in Biochemical Components of the Visceral Mass and Adductor Muscle in the Pen Shell, *Atrina pectinata*

Sung-Hyun Baik, Kang-Jeon Kim, Ee-Yung Chung
Jong-Jae Choo[1] and Kwan Ha Park*
Faculty of Marine Life Science and
[1]Department of Foods & Nutrition, Kunsan National University,
Kunsan City, Chonbuk 573-702, Korea

(Received January 2001, Accepted March 2001)

Seasonal content changes of the three key nutrients for organisms, protein, lipid and glycogen, were analyzed for a whole year to delineate the seasonal energy strategy in pen shells, *Atrina pectinata*. Two metabolically important organs, the visceral mass and the posterior adductor muscle, were examined. Protein in the visceral mass rose in April and stayed at the level until June followed by the constant minimum value between August and November. The protein contents in the posterior adductor muscle increased sharply in April and again in July, followed by a gradual decline thereafter. Total lipid contents in the visceral mass gradually increased between January and May, and then slowly decreased until September since which a new weak increase was noticed. Lipid levels in the adductor muscle rapidly dropped in June and July. Glycogen contents in the visceral mass rapidly increased between February and June, followed by a drastic drop in July. This reduced visceral glycogen level was maintained up to September, and a gradual reduction ensued. Glycogen contents in the adductor muscle steadily but markedly increased from April reaching the maximum in August, and then slowly declined thereafter. These results suggest that an accelerated protein and lipid synthesis occurs in the gonad when the pen shell undergoes the ripe stage of gametogenesis, but the levels of these two nutrients decrease on spawning. With this gonadal process, regular protein synthesis and lipid storage in the posterior adductor muscle are temporarily arrested. The most important nutrient reserves that support gonad developmental cycles in a long term seem to be glycogen of the posterior adductor muscle.

Key words: Pen shell, Protein, Lipid, Glycogen, Gametogenesis

Introduction

The pen shell, *Atrina pectinata*, is one of the biggest bivalves inhabiting limited areas of the Southern and Western Coast of Korea (Kwon et al., 1993). Due to its delicace taste it is considered a highly valuable seafood in some Asian countries. As the whole harvest has been solely dependent on wild pen shells, yearly production has been declining for the past decades. *A. pectinata* is found in 15~50 cm deep silts at 20~50 m off the coasts. Some studies have dealt with its ecology (Yoo and Yoo, 1984), artificial culture techniques (Yoo et al., 1986) and genetic divergence (Yokokawa, 1996).

Although the pen shell is a valuable marine species for possible aquaculture industry, sufficient information on its biology is clearly lacking. In particular, no information is available on its seasonal variations in biochemical compositions. If any, data might have been obtained to evaluate its nutritional value as food, usually examining only samples obtained during harvest seasons (Ha, 1989). Information on the processes of energy supply and storage during growth and reproduction will serve a keystone role for un-

*Corresponding author: khpark@ks.kunsan.ac.kr

derstanding the general biology of this industrially valuable species. In this study, we analyzed biochemical component changes of pen shells collected for a period of one year from the subtidal area on the Western Coast of Korea. Discussion was made on the significance of seasonal biochemical variations in conjunction with gonad development throughout a year.

Materials and Methods

Sample preparations

Pen shells were collected by scuba diving from the coast of Nokdo Island, Boryong City, Chungnam, Korea, from January through December 1999. For each month enough numbers of samples of 23~27 cm long were collected and 10 female individuals were used for the study. Collected samples were frozen immediately and transported to the laboratory and kept in a deep-freezer (-70℃) until analysis. On thawing, both the visceral mass which included the gonad and digestive diverticular, and the posterior adductor muscle were separately removed. Removed tissues were blotted with a paper towel and all biochemical analyses were done on wet tissues.

Protein analysis

About 50 mg tissues were homogenized in 4 volumes of physiological saline. One hundred μL of homogenate was diluted to 1.0 mL in glass tubes with distilled water and 1.0 mL Lowry reagent (Lowry et al., 1951) was added, then incubated for 20 min at room temperature. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the tube and absorbance was measured at 750 nm (Shimadzu UV-1601PC) following 30 min incubation at room temperature, using bovine serum albumin as a standard.

Lipid analysis

Tissue of about 1 g was homogenized with 50 mL of 100% methanol. Chloroform (50 mL) was added and the homogenate was filtered after keeping at room temperature for 60 min. The remnant on a filter paper was further extracted 3~4 times with a 10~20 mL portions of chloroform and all filtrate was pooled. The filtering assembly was rinsed with 20 mL of methanol-chloroform (1:1) mixture and the rinsed mixture was added to the filtrate. A volume of 0.5% $ZnSO_4$ solution corresponding to 40 % of total chloroform used was added and the mixture was vigorously shaken in a separatory funnel. The lower phase was retrieved and evaporated in a rotary evaporator at 30℃ under vacuum. Total lipid content was calculated by weighing the residue following desiccation.

Glycogen analysis

Appropriate amount of excised tissues (~1 g) was placed in test tubes and 2 mL of 30% KOH solution was added, followed by heating for 2 hrs in boiling water. After cooling on ice, 4 mL of 95% ethanol was added and incubated in a refrigerator overnight. The solution was centrifuged at 5,000×g for 10 min to precipitate glycogen. The precipitate was dissolved in 2 mL of 2% Na_2SO_4 and the supernatant was discarded after centrifugation (5,000×g, 10 min). The precipitate was treated with 6 mL of 1 N H_2SO_4 and the resulting supernatant was used for colorimetry. Five mL of anthrone-H_2SO_4 reagent was added and heated for 15 min in boiling water followed by cooling for 20~30 min at room temperature. Developed color was quantified spectrophotometrically at 620 nm using D-glucose solutions as standards.

Data expression

All data were express as mean±SEM. Statistical significance among mean values was usually not analyzed except for the cases specified in the text, because our interest was focused on understanding the trend of seasonal variations related to gonadal development.

Results and Discussion

Reproductive cycle with gonadal development

Reproductive cycles are operated based on the build-up of gametes and their release under the condition that is favorable for larval survival and growth (Strohmeier et al., 2000). The seasonality in temperature and food availability thus results in seasonal cycles in somatic nutrient storage that will be utilized later during food shortage or for gametogenesis. It has also been suggested that the seasonal change in somatic growth and gametogenesis is attributable to the competition for energy reserve and available food resources (Pearse et al., 1986).

In many bivalves, it has been reported that there is a seasonal biochemical variation in the gonad or adductor muscle that can be closely correlated with reproductive activities. For example, increases in protein and lipid contents in the gonad during gonadal developmental periods, and also an increase in carbohydrates on spawning were reported for *Mytilus edulis* (Williams, 1969; Bayne and Thompson, 1970). A similar variation was also noted in *Musculium transversum* (Dietz and Stern, 1977). Differently from these two species, Ansell et al. (1964) reported that carbohydrate contents increased during gonadal proliferation in the *Mercenaria mercenaria* gonad. Increases in glycogen and protein contents in the queen scallop (*Chlamys opercularis*) adductor muscle are associated with increasing food abundance and accompanied gonadal maturation (Taylor and Venn, 1979). In *Chlamys septemradiata*, glycogen and protein reserved in the adductor muscle are depleted during gonadal development (Ansell, 1974). The adductor muscle of *Pecten maximus* stores energy as glycogen and protein when energy supply is sufficient and uses for gametogenesis (Comely, 1974).

It has been demonstrated that there exists a distinctive seasonal cycle in the gonadal development for reproduction in the pen shell, *Atrina pectinata* (Baik, 1998). Gonad index of the pen shell rapidly increases between February and April in response to the elevation of seawater temperature. The index then declined dramatically with spawning between May and September, followed by a slow decrease. Baik (1998) was able to classify reproductive cycle of this bivalve species into five distinct stages based on histological examinations of the gonad (Table 1). To supplement required energy in pen shell for the cycle operation, massive energy mobilization and consumption of stored nutrients might be expected.

Although there have been some studies on seasonal changes of biochemical composition in other marine bivalves (Walne, 1970; De Zwaan and Zandee, 1972; Ansell, 1974; Comely, 1974; Taylor and Venn, 1979; Kang et al., 2000; Strohmeier et al., 2000), no literature has directly dealt with nutritional variations of the pen shell in response to gonadal maturation. In this study, we analyzed the major nutritional contents of pen shell to focus on two important soft tissues: (1) the visceral mass which is mostly composed of the gonad and partly of the digestive diverticula; and (2) the posterior adductor muscle which may be most important in its size and energy capacity for nutrient supply in a longer term.

Table 1. Gonadal stage of the pen shell, *Atrina pectinata*, collected from the subtidal zone of Nakdo, Chungnam, Korea (modified from Baik, 1998).

Gonadal phase	Months
Early developing stage	November-March
Late developing stage	February-April
Ripe stage	February-August
Partially spawned stage	May-September
Spent/Resting stage	July-January

Total protein contents

Protein contents in the visceral mass increased in April and stayed at that level for 2~3 months followed by a gradual decline in June and July (Fig. 1). The visceral protein remained at lower values between August and November, and then a rising tendency appeared in December. The adductor muscle protein levels were at a declining trend from January to March (Fig. 2), but the decline was converted to a rise in April. The rise was then interrupted in May and June remaining at constant levels. There was a second marked rise in the adductor muscle protein in July followed by a gradual decrease through the end of the year.

The sharp increase of visceral protein in April may

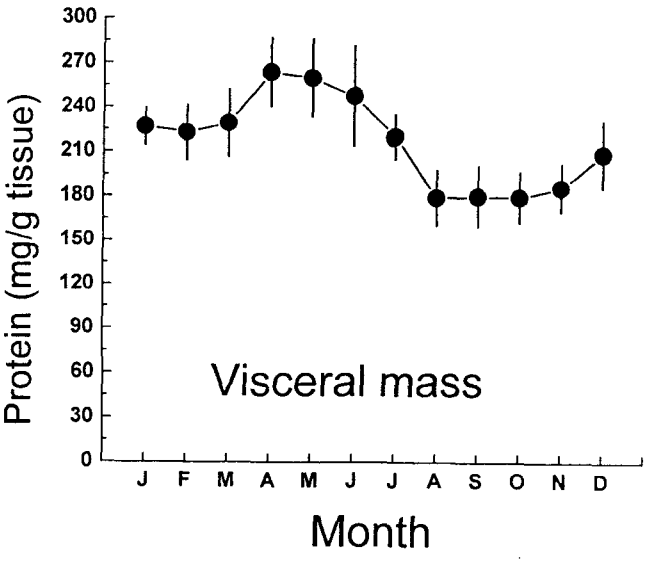

Fig. 1. Monthly protein content changes in the visceral mass of pen shells (n=10).

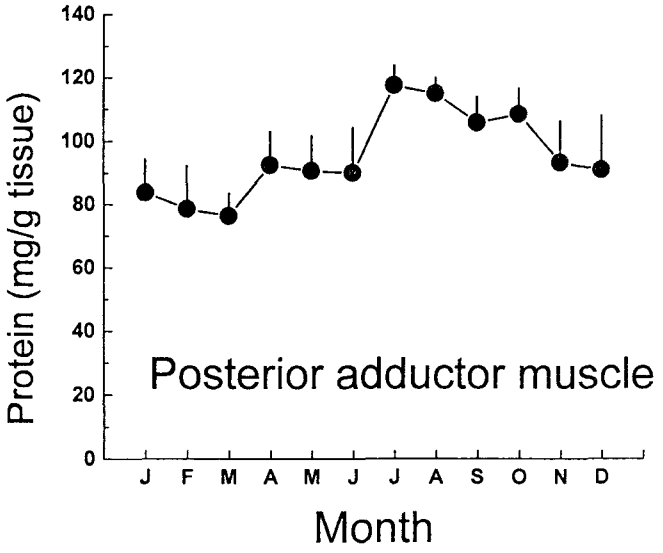

Fig. 2. Monthly protein content changes in the posterior adductor muscle of pen shells (n=10).

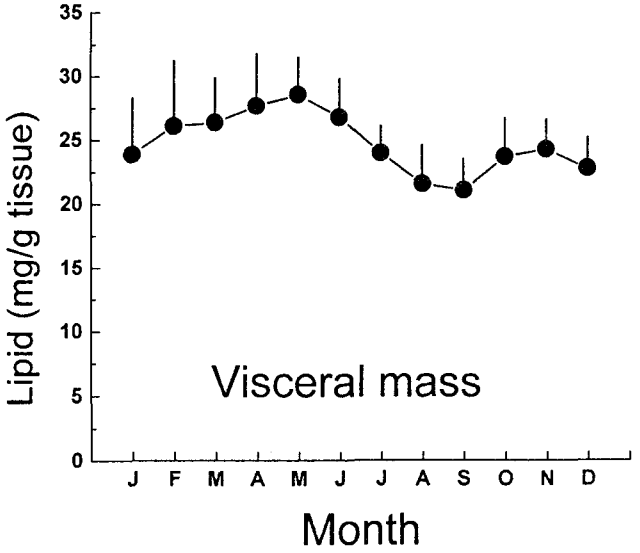

Fig. 3. Monthly lipid content changes in the visceral mass of pen shells (n=10).

indicate a burst protein synthesis required for oogenesis. It has been shown that most pen shells collected in April from the study were at the "ripe stage", and that only a few at the "late developing stage" (Baik, 1998; also refer to Table 1). The number of larger eggs is most abundant in April (Baik, 1998). Thus the observed peak protein levels of visceral mass in April may be indicative of this accelerated gamete maturation in the female gonad. There were increases in protein contents initially in April and again in July in the adductor muscle, but a break in the increasing tendency was apparent in May and June. Protein represents primarily major structural part of the adductor muscle, although it can also be utilized to a certain extent to support gametogenesis or for maintenance purpose in bivalves (Barber and Blake, 1991). Protein levels in the muscle is also an indicator of somatic growth, and therefore the increase from May through July could suggest normal somatic growth arising from available food abundance. However, it seems that the growth might have been halted between May and June because significant portion of energy resources was utilized for gametogenesis.

Total lipid contents

The lipid content changes in the visceral mass are shown Fig. 3. The change was somewhat inconspicuous although their trends were similar to those of visceral protein in general. The highest and lowest mean levels (not statistically significant, $p>0.05$ with unpaired t-test) respectively appeared in May and September. In contrast to the low sensitivity of visceral lipid change, its level in the posterior adductor muscle suddenly dropped in June and July (Fig. 4).

Lipid levels in the gonad are expected to rise in the process of gametogenesis, and to decrease during spawning in bivalves (Gabbott, 1976; Lubet, 1976; Gabbott, 1983). The variations of lipid changes, i.e., the maximum level in May and subsequent decline

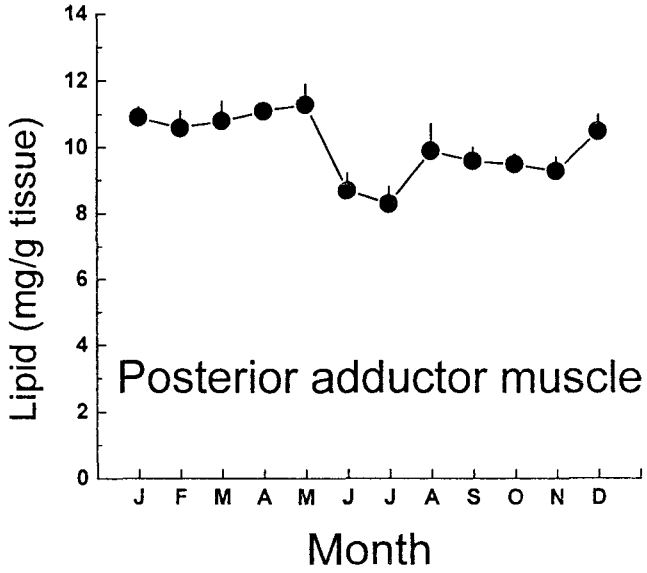

Fig. 4. Monthly lipid content changes in the posterior adductor muscle of pen shells (n=10).

thereafter, in the visceral mass seem to reflect thus the gonadal maturation and spawning. Lipid storage occurs as an energy reserve when food supply exceeds utilization (Pollero et al., 1979). A marked decrease was observed in the lipid level of adductor muscle in May to July. There is no good explanation for this sudden lipid drop at present, but it is likely that most nutrient resources may have been diverted to accelerated protein or glycogen biosynthesis.

Despite the relative insignificance of visceral lipids, compared to glycogen (see below) or protein, the present findings agree well with the those from other bivalves. For example, accumulation of gonadal lipid is prominent prior to gametogenesis and its utilization is related to the initiation of oogenic activity in *Agropecten irradians* (Barber and Blake, 1981). It has also been demonstrated that lipid material accumulates in the sea mussel connective tissue during the period of gonadal development to serve as a nutrient pool for the growing gametes (Chipperfield, 1953; Williams, 1969; Bayne and Thompson, 1970).

Glycogen contents

Glycogen in the visceral mass began to increase sharply from March and the maximum level was attained in June, then it declined rapidly in July (Fig. 5). The declined level was maintained until September, but was followed by a further reduction between September and November. In the posterior adductor muscle, glycogen level rose markedly from April reaching the maximum in August (Fig. 6). After that, it steadily decline until December.

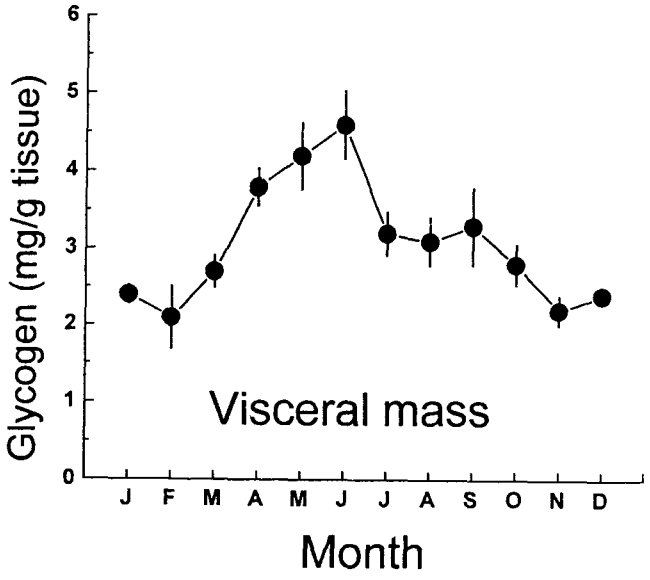

Fig. 5. Monthly glycogen content changes in the visceral mass of pen shells (n=10).

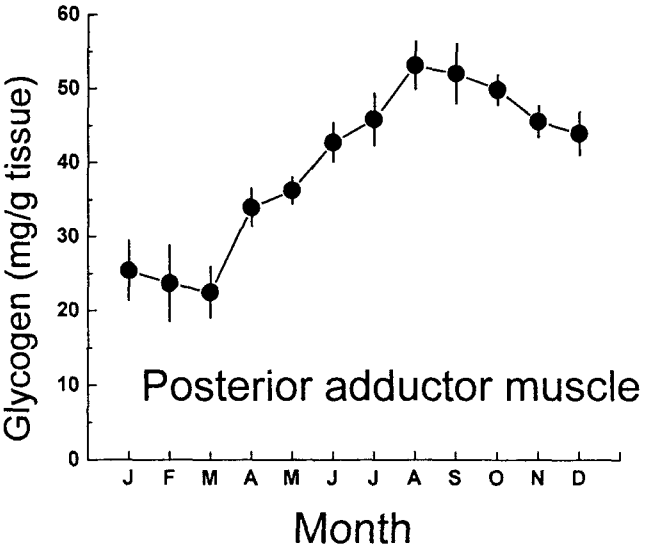

Fig. 6. Monthly glycogen content changes in the posterior adductor muscle of pen shells (n=10).

Generally in bivalves, energy is stored the form of glycogen for an immediate utility when food supply is abundant, and is utilized for the production of gametes during which energy demand is high (Gabbott, 1975; Bayne, 1976). Gabbott (1975) described that a synchronous change was found between glycogen utilization and oogenesis in Mytilus edulis. Barber and Blake (1983) also observed that reserve energy was stored in the adductor muscle as glycogen substrates and depleted during oocyte maturation and vitellogenesis.

Our finding that the glycogen content in the visceral mass increased between March and June as the gonadal tissues underwent the ripe stage (Baik, 1998). Furthermore, the rapid drop in July seems to reflect the spawning event in which most of the ripe oocytes are discharged. The lowest visceral glycogen level from November to February may be related to "spent or resting stage" at which gonadal remnants are absorbed, and new oogenic follicles begin to appear.

The consistent accumulation in adductor muscle glycogen between April and August indicates that

there has been abundant food resources like phytoplanktons when the seawater temperature rose (Ansell, 1974). Glycogen reserves in the adductor muscle is the main energy source under the condition of high nutritional needs such as gametogenesis or predator avoidance (Ansell, 1974; de Zwaan et al., 1980). It is however likely that the immediate environmental food resources required for pen shell gametogenesis was sufficient, so that the energy storage process in the adductor muscle as glycogen might not be apparently interrupted except in March.

Gonadal development and energy strategy in the pen shell

The significance of adductor muscle as an energy reserve organ demonstrated in other bivalves seems to be valid in the pen shell. In the adductor muscle, the protein content rise was temporarily stopped when spawning started in May and June, and the lipid content also dropped rapidly slightly later in June and July when most pen shells underwent spawning. Glycogen accumulation occurred in the adductor muscle from April to August, during which abundant food must have been available. This stored energy will be utilized for the gonadal development starting from November (Baik, 1998).

The interpretation of nutritional level changes in the posterior adductor muscle is straightforward as this organ may exclusively serve as a storage organ/supply site: increase and decrease respectively indicates saving and utilization. However, the understanding of change in the visceral mass needs caution since the change in this tissue is the net result of both from the gonad and the remaining digestive tissues. The apparent visceral mass level therefore is dependent on the one which is dominant in its change. However, the biochemical changes in the visceral mass suggest that changes in the gonad, not the remaining tissues, seem to have largely contributed to the presently observed variations.

Acknowledgements

The work was supported by the RRC Program of MOST and KOSEF through the Saemankeum Environmental Research Center in Kunsan National University.

References

Ansell, S.D. 1974. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea area. Mar. Biol., 25, 85~99.

Ansell, A.D., F.A. Loosmore and K.F. Lander. 1964. Studies on the hard-shell clam, *Venus mercenaria,* in British waters. II. Seasonal changes in condition and biochemical composition. J. Appl. Ecol., 1, 83~95.

Baik, S.H. 1998. Studies on reproduction and growth of the pen shell, *Atrina (Servatrina) pectinata,* on the West Coast of Korea. Master's Thesis, Kunsan National University (in Korean).

Barber, B.J. and N.J. Blake. 1983. Growth and reproduction of the bay scallop, *Argopecten irridians* (Lamarck) at its southern distributional limit. J. Exp. Mar. Ecol., 66, 247~256.

Barber, B.J. and N.J. Blake. 1991. Reproductive physiology. In *Scallops:* Biology, ecology, and aquaculture. S.E Shunway eds., Elsevier, Amsterdam, pp. 377~428.

Bayne, B.L. 1976. Aspects of reproduction in bivalve molluscs. In *Esturine Processes,* M.L. Wiley ed., Academic Press, New York, pp. 432~448.

Bayne, B.L. and R.J. Thompson. 1970. Some physiological consequences of keeping *Mytilus edulis* in the laboratoy. Helgol. Wiss. Meeressunters., 20, 626~552.

Chipperfield, P. 1953. Observation on the breeding and settlement of *Mytilus edulis* (L.) in British waters. J. Mar. Biol. Assoc. U.K., 32, 449~476.

Comely, C.A. 1974. Seasonal variations in the flesh weights and biochemical content of the scallop *Pecten maximus* L. in the Clyde Sea Area. J. Cons. Perm. Int. Explor. Mer., 35, 281~295.

De Zwaan, A., R.J. Thompson and D.R. Livingstone. 1980. Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten magellanicus.* II. Biochemistry. J. Comp. Physiol., 137, 105~114.

De Zwaan, A. and D.J. Zandee. 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis.* Comp. Biochem. Physiol. A, 43, 53~58.

Dietz,T.H. and E.M. Stern. 1977. Seasonal changes in reproductive activity and biochemical composition of the fingernail clam, *Sphaerium transversum.* Nautilis, 91, 136~140.

Gabbott, P.A. 1975. Storage cycles in marine bivalve molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In *Marine Mussels,* B.L. Bayne ed., Cambridge Univesity Press, Cambridge, pp. 293~355.

Gabbott, P.A. 1976. Energy metabolism. In *Marine Mussels:* Their ecology and physiology. B.L. Bayne ed., Cambridge University Press, Cambridge, pp. 121~126.

Gabbott, P.A. 1983. Developmental and seasonal metabolic activities in marine molluscs. In *The Mollusca.* vol. 2. A. S.M. Saleuddin and K.M. Wilber ed., Academic Press, New York, pp. 165~217.

Ha, S.H. 1989. Chemical composition of marine products in Korea. Utilization Research Laboratory, National Fisheries Research and Development Agency, Korea (in Korean).

Kang, C.K., M.S. Park, P.Y. Lee, W.J. Choi and W.C. Lee. 2000. Seasonal variations in condition, reproductive activity, and biochemical composition of the Pacific oyster, *Crassostrea gigas* (Thunberg), in suspended culture in two coastal bays of Korea. J. Shellfish Res., 19, 771~778.

Kwon, O.K., K.M. Park and J.S. Lee. 1993. Coloured Shells of Korea. Academy Publishing Co., Seoul, Korea. 445 pp. (in Korean).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.L. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193, 265~275.

Lubet, P. 1976. Ecophysiologie de la reproduction chez lez les mollusques lamellibranches. Haliotis, 7, 49~55.

Pearse, J.B., V.B. Pearse and K.K. Davis. 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. J. Exp. Zool., 237, 107~118.

Pollero, R.J., M.E. Re and R.R. Brenner. 1979. Seasonal change of the lipids of the mollusc *Chlamys tehuelcha*. Comp. Biochem. Physiol. A, 64, 257~263.

Strohmeier, T., A. Duinker and ϕ. Lie. 2000. Seasonal variations in chemical composition of the female gonad and storage organs in *Pecten maximus* (L.) suggesting that somatic and reproductive growth are separated in time. J. Shellfish Res., 19, 741~747.

Tayler, A.C. and T.J. Venn. 1979. Seasonal variation in weight and biochemical composition of the tissues of the queen scallop, *Chlamys opercularis*, from the Clyde Sea Area. J. Mar. Biol. Ass. U.K., 59, 60 5~621.

Walne, P.R. 1970. The seasonal variation of meat and glycogen content of seven populations of oysters *Ostrea edulis* L. and a review of the literature. Fish. Invest., Lond., (Ser. II), 26, 35.

Williams, C.S. 1969. The effect of *Mytilicola intestinalis* on the biochemical composition of mussels. J. Mar. Biol. Assoc. U. K., 49, 161~173.

Yokogawa, K. 1996. Genetic divergence in two forms of pen shell, *Atrina pectinata*. Japan. J. Malacol., 55, 25~39.

Yoo, S.K., H.S. Lim, H.J. Ryu and K.H. Kang. 1986. Improvement of the seed production method of the pen shell: The occurrence of larvae and the early growth of the spat. Bull. Kor. Fish. Soc., 21, 206~216.

Yoo, S.K. and M.S. Yoo. 1984. Studies on the pen shell culture development I: Reproductive ecology of pen shell in Yoja Bay. Bull. Kor. Fish. Soc., 17, 529~535.