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Changes in Biochemical Composition of the Digestive Gland of the Female Purple Shell, *Rapana venosa*, in Relation to the Ovarian Developmental Phases

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ABSTRACT

The Ovarian developmental phases of the reproductive cycle of Rapana venosa can be classified into five successive stages by histological study: early active stage (September to February), late active stage (December to April), ripe stage (March to July), partially spawned stage (May to August), and recovery stage (June to September). To understand the characteristics of nutrient storage and utilization in the digestive gland cells with ovarian developmental phases, we examined the digestive gland - which is the major nutrient supply organ associated with ovarian development of the female purple shell - by biochemical methods.

Total protein contents in the digestive gland tissues increased in March (late active stage) and reached the maximum in May (ripe and partially spawned stages), and then their levels sharply decreased in July (partially spawned and recovery stages). Total lipid contents in the digestive gland tissues reached the maximum in January (early active stage). Thereafter, their levels rapidly decreased from May (ripe and partially spawned stages) and reached a minimum in July (partially spawned and recovery stages). The total DNA contents did not significantly change regardless of the different developmental stages of the ovary. However, it was also found from biochemical analysis that changes in total RNA content follow the same seasonal cycling to protein. These results indicate that the digestive gland is an important energy storage and supply organ in purple shells, and that the nutrient contents of the digestive gland change in response to gonadal energy needs.

Keywords: *Rapana venosa*, Ovarian development, digestive gland, Total protein, Total lipid, Nucleic acid contents.

INTRODUCTION

The purple shell, *Rapana venosa* (Gastropoda: Muricidae), which is one of the most important edible gastropods (Yoo, 1976; Kwon *et al.*, 1993), is abundantly present along the coasts of Korea, China and Japan in silty sand of the intertidal and subtidal zones. It is well-known that the digestive gland is the major nutrient storage and supply organ to the gonads for germ cell development. For the study of their physiological roles and relationship between nutrient storage and nutrient supply of the digestive gland to the gonads, it is very important to investigate seasonal changes of biochemical compositions in the digestive gland tissues connected to the gonads.

Previously there have been some studies on reproductive ecology (Hirase, 1928; Kuroda and Habe, 1952; Amio, 1963; Chung *et al.*, 1993; Chung and Kim, 1997), morphology (Lee and Kim, 1988), molecular biology (Yoon, 1986), and heavy metal pollution (Yoo *et al.*, 1991) of the purple shell. However, there is still no information on the changes

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of biochemical components in the digestive gland tissues with ovarian developmental phases of purple shells. The present study was aimed to trace biochemical component changes of the digestive gland tissue with the ovarian developmental phases of this species.

MATERIALS AND METHODS

1. Sampling

Samples of the purple shell, *Rapana venosa*, were collected monthly by dredging at the subtidal zone near Bieungdo, Jeollabuk-do, Korea, from May 1998 to May 1999. Shells ranging from 11.0-12.4 cm in shell height were used for the present study. After the live purple shells were transported to the laboratory, the specimens were used for histological and cytological studies (Fig. 1).

2. Histological study of ovarian development

Histological preparations were made for analysis of the gonadal phases by light microscopy. The gonad tissues were separated from the shells and preserved in Bouins fixative for 24 h and then washed with running tap water for 12 h. Tissues were then dehydrated in ethanol, embedded in paraffin and sectioned at 5-7 μm in thickness using a rotary microtome. Sections were mounted on glass slides, stained with either Hansen's haematoxylin-0.5% eosin, Mallory triple stain or PAS (Periodic Acid-Schiff) stain, and examined under a light microscope. Examination of gonad variability in R. venosa showed no significant differences in the reproductive state between 3 random sections taken from different positions in the gonad of each sex (Fig. 1B). Sections were assigned to one of five stages: 1) early active, 2) late active, 3) ripe, 4) partially spawned, and 5) recovery stage, based on the minor modifications of the staging criteria used by Redfern (1974). Two or more stages often occurred simultaneously within each section, therefore, the staging criteria decisions were based upon the conditions of the majority of the section.

3. Biochemical analysis of the digestive gland tissues

Whole digestive gland tissues were used for the estimation of nutritional utilization. The specimens representing characteristics of ovarian developmental phases were collected in March, May, July and September. Tissues were dissected on ice and frozen immediately until analysis at -80℃. Wet digestive gland tissue samples were weighed after a brief

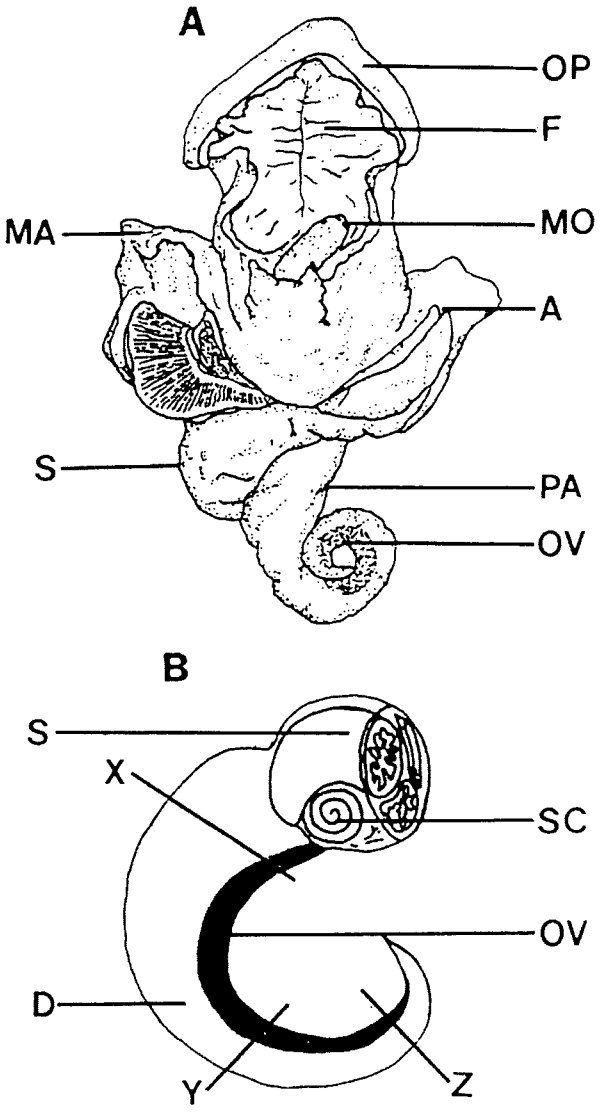

Fig. 1. Anatomy of the female purple shell, *Rapana venosa*, removed from its shell.
A, reproductive organ of female; B, posterior appendage showing the gonad and digestive gland. X, Y and Z denote the sections for confirmation of the gonadal development. Three sections are spaced equality. Abbreviations: A, anus; D, digestive gland; F, foot; MA, mantle; MO, mouth; OP, operculum; OV, ovary; PA, posterior appendage; S, stomach; SC, stomachal caecum.

blotting with a paper towel. Samples were homogenized in phosphate-buffered saline solution (pH 7.4) at 4 ℃ using a glass homogenizer, and then further broken down with an ultrasonicator (Braum-Sonic 1510, 10 min). After centrifugation at 3,000 x g for 10 min (2℃), cellular debris was discarded.

Total protein content was analyzed with a minor modification of Lowry procedures (Lowry *et al.*, 1951). In brief, the supernatant was mixed with $CuSO_45H_2O$ solution (Reagent B) along with Na_2CO_3 (Reagent A) and potassium tartrate (Reagent C) solutions. The mixture was reacted with 2N Folin-phenol reagent and the color developed was measured using a spectrophotometer (UV-120-02, Shimadzu, Japan) at 660 nm.

For total lipid content analysis, the digestive glands were desiccated in a drying oven at 50℃ for 3 hrs. Tissues of about 20 g were placed in a Soxhlet apparatus thimble and lipids were extracted with ether for 48 hrs at 50℃. Ether was evaporated with a rotary evaporator and total lipid content was calculated by weighing the amount left.

Extraction and quantification total RNA and total DNA was carried out according to the methods of Munro and Fleck (1969). Homogenized tissues of about 250 mg were precipitated with a 0.22 N perchloric acid (PCA) solution. Samples were then stored in ice-cold bath for 20 min, centrifuged at 3,000 g (2℃) for 10 min, and the supernatant was discarded. The precipitate was dissolved in 4 ml 0.3 N KOH solution by incubating in warm (37℃) water for 2 hr. One ml of 2.3 N PCA was added to the sample, and centrifuged at 3,000 g (2℃) for 20 min to separated RNA (supernatant) from DNA (precipitate) fraction. For RNA analysis, a 2 ml orcinol reagent was added to 1 ml of the supernatant, heated in boiling water for 20 min, and the developed color was quantified with a spectrophotometer at 665 nm. The precipitated DNA was dissolved in 5 ml 0.3 N KOH by heating for 20 min. A 3 ml indole reagent was added to 1 ml dissolved DNA solution and the sample was again heated for 20 min. The sample was washed with 4 ml chloroform and centrifuged at 3,000 x g for 10 min, and the DNA quantity was measured with a spectrophotometer at 490 nm. The standards for content determination for RNA and DNA were from processed bulk yeast and calf serum thymus (Sigma), respectively.

4. Statistics

Data from multiple measurements are expressed as mean ± SE. Data were analyzed by a one-way analysis of variance followed by the Duncan test. The criterion for statistical significance was $p < 0.05$.

RESULTS

1. Reproductive cycle with ovarian developmental phases

Based on morphological characteristics and differentiation of germ cells and tissue cells around them by histological observations, the ovarian phases can be classified into five successive stages and show a periodicity. Principal histological characteristics of ovarian developmental phases are as follows:

Early active stage

The early active stage was characterized by the expansion of the follicle and the appearance of oogonia measuring about 15 μm in diameter and early developing oocytes ranging 60-70 μm in diameter along the follicular wall (Fig. 2A). The individuals in the early active stage appeared from September to February.

Late active stage

Walls of the follicles were thin and the connective tissue decreased during this stage. A number of early developing oocytes of 120-140 μm in diameter formed an egg-stalk attached to the follicular wall (Fig. 2B). With yolk formation, there are numerous yolk granules in the cytoplasm of late developing oocytes ranging 150-190 μm in diameter. Several fully ripe oocytes were free in the lumen of the follicle (Fig. 2C).

The individuals in the late active stage were found from December to April.

Ripe stage

The ripe ovary showed distended follicles with detached mature and fully ripe oocytes. The follicles

occupied over 65% of the ovary and follicular walls became very thin. Mature oocytes which had grown to 230-250 μm in diameter became polygonal in shape and contained a number of mature yolk granules (Fig. 2D). The individuals in the ripe stage appeared from March to July.

Partially spawned stage

Free ripe oocytes in the lumen of the follicle decreased by about 50%, and empty follicles appeared. This stage was characterized by the presence of several undischarged oocytes in the follicle (Fig. 2E). The individuals in the partially spawned stage were found from late May to August.

Recovery stage

After spawning, follicles shrunk, and then degeneration or reabsorption of the oocytes occurred. Thereafter, connective tissues and newly formed oogonia appeared on the follicular wall (Fig. 2F). The

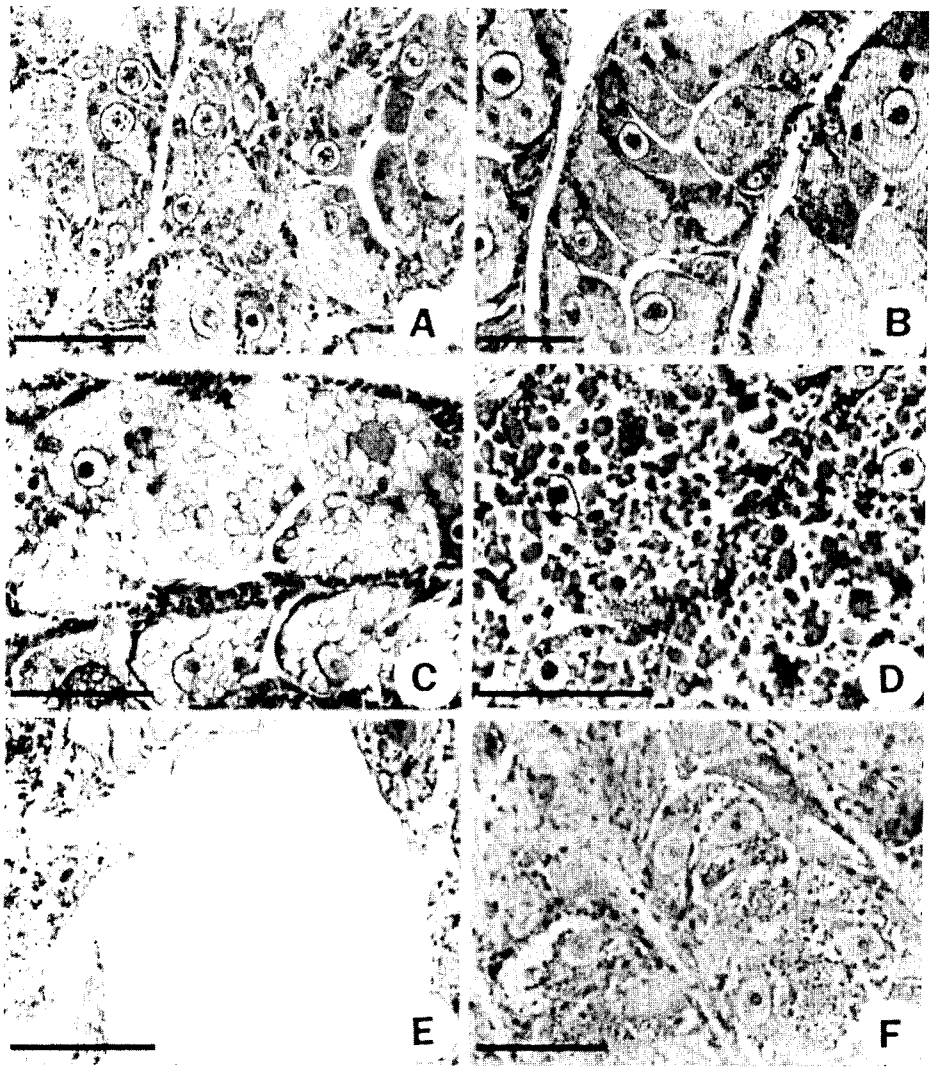

Fig. 2. Photomicrographs of ovarian developmental phases of the female purple shell, *Rapana venosa*.
A, Transverse section of the follicles in the early active stage, scale bar = 100 μm; B, C, sections of follicles in the late active stage, scale bars = 100 μm, 100 μm ; D, section of follicles in the ripe stage, scale bar = 100 μm; E, section of follicles in the partially spawned stage, scale bar = 100 μm; F, section of follicles in the recovery stage, scale bar = 100 μm.

individuals in the recovery stage were found from June to September.

2. Changes in biochemical contents of the digestive gland tissues

From the results of electron microscopical observations and biochemical analysis, it was confirmed that the digestive gland tissues were the nutrient storage organ or the supply organ for gonadal development of gastropods, and the biochemical composition of various substrates in the digestive gland tissues changed to contribute to energy metabolism during gonadal development. Changes in total protein, total lipid, total DNA, and total RNA contents in relation to ovarian developmental phases as shown by biochemical analysis were as follows:

Total protein

Changes in total protein contents of the digestive gland with ovarian developmental phases are shown in Table 1. Total protein content increased in March (late active stage) and reached the maximum in May (ripe and partially spawned stages). But, their levels sharply fell to the minimum value in July (partially spawned and recovery stages) and increased slightly in September (recovery and early active stages). Statistical analysis revealed that the content in each month was significantly different from those of the other three months at $p < 0.05$ (Duncan test).

Total lipid

Changes in total lipid content of the digestive gland with ovarian developmental phases are shown in Table 2. The lipid content became the highest in March (late active stage), and levels decreased sharply in May (ripe and partially spawned stages), reaching the minimum in July (partially spawned and recovery stages). It was found that lipid levels were statistically different ($p < 0.05$, Duncan test) for each of the five months tested.

Total DNA and total RNA

As shown in Table 3, the total DNA content did not significantly change regardless of the different developmental stages of the ovary. The total RNA content, in contrast, fluctuated depending on the stage. Total RNA levels were high in March (late active stage) and reached the maximum in May (ripe and partially spawned stages). Thereafter, their contents significantly decreased in July (partially spawned and recovery stages) and gradually increased in September (recovery and early active stages).

Table 1. Total protein content of the digestive gland of the female purple shell, *Rapana venosa*.

Month	Protein content (mg/g)	Gonadal phase
March	62.46 ± 8.82	Late active stage
May	66.34 ± 9.04	Ripe and partially spawned stage
July	38.53 ± 6.32*	Partially spawned and recovery stages
September	42.77 ± 8.84	Recovery and early active stages

*Significantly different from March and May ($p < 0.05$ with Duncan test, $n = 10$).

Table 2. Total lipid content of the digestive gland of the female purple shell, *Rapana venosa*.

Month	Total lipid content	Gonadal phase
January	154.32 ± 16.42	Early active stage
March	143.54 ± 15.54	Late active stage
May	157.94 ± 18.71*†	Ripe and partially spawned stages
July	153.37 ± 16.89*†	Partially spawned and recovery stages
September	170.77 ± 17.81*†	Recovery and early active stages

Significant difference from January (*) and March (†) at $p < 0.05$ with Duncan test ($n = 10$).
Each determination consisted of pooled digestive glands from 10 individuals.

DISCUSSION

In this study we investigated the relationship between ovarian development and mobilization of nutrient materials in the digestive gland cells of the female purple shell, *Rapana venosa*, using biochemical methods.

Table 3. Total DNA and RNA content of the digestive gland of the female purple shell, *Rapana venosa*.

Month	Total DNA (mg/g)	Total RNA (mg/g)	Gonadal phase
March	7.29 ± 0.61	8.37 ± 0.92	Late active stage
May	7.26 ± 1.13	9.07 ± 0.94*	Ripe or spawning stages
July	7.23 ± 0.91	7.64 ± 0.80*†	Spawning or recovery stages
September	7.24 ± 0.92	7.91 ± 0.83†	Recovery or early active stages

Significant difference from March (*) and May (†) at $p < 0.05$ with Duncan test (n = 10).
No significance difference in total DNA contents was found among groups.

Biochemical study also showed that total protein content rapidly began to increase in March (late active stage) and reached the maximum in May (ripe and spawning stages). Thereafter, total protein content gradually decreased from July through September. These results suggest that seasonal variations of total protein content temporally coincide with those of total RNA content. Barber and Blacke (1981) stated that variations of total protein content in the digestive diverticula of *Argopecten irradians* show the maximum during the period of gonadal development and maturation, and their content rapidly decreases after the spawning period. Therefore, our results with R. venosa agree with the opinion of Barber and Blacke (1981). In the case of bivalves, the biochemical composition of various substrates in scallops change to contribute to energy metabolism during gonadal development (Barber and Blacke, 1981). According to the results in the present study, digestive gland lipid contents were relatively low prior to gonadal development in September. With the initiation of gonadal development, however, the lipid content reached the maximum in January (early active stage) through March (late active stage) followed by a decrease in May when gametes matured (ripe stage).

Mori (1975) found that digestive gland lipid decreased prior to gonadal development in *Patinopecten yessoensis*. Robinson *et al.* (1981) also observed that digestive gland lipids and carbohydrates were depleted, whereas gonadal lipid content reached a maximum as gametes matured in *Placopecten magellanicus*. According to Barber and Blacke (1981), prior to gametogenesis, nutrients were stored in the digestive gland and adductor muscles in *Argopecten irradians*. The utilization of lipid from the digestive gland was associated with the initiation of oocyte growth, vitellogenesis and the corresponding increases in gonadal protein and lipid levels.

In this study we monitored the seasonal changes of ovarian development in the gastropod (*Rapana venosa*) by histological examination of the digestive gland. Our results of quantitative variations biochemically confirmed by analysis of nutritional components coincide with the results of Barber and Black (1981).

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