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GERM CELL DIFFERENTIATION AND SEXUAL MATURATION OF THE FEMALE *Neptunea cumingii* (GASTROPODA: BUCCINIDAE)

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INTRODUCTION

Neptunea cumingii is one of the most important edible gastropods in East Asian countries such as in Korea, Japan, Russia, and the other countries. This species is especially found in silty sand of the subtidal zone of the west coast of Korea. Recently, as the standing stock of this species gradually decreased due to extensive reclamation projects and reckless over-harvesting, it has been designated as one of the important organisms in need of natural resources management. On *Neptunea* spp. in foreign countries and Korea, previously, there have been some studies on aspects of reproduction including the reproductive cycle (Takahashi et al., 1972; Takamaru & Fuji, 1981; Fujinaga,1985) and spawning (Miyawaki, 1953), on aspects of ecology including distribution, growth of *Neptunea arthritica*, and feeding of *N. antique*. Little information is available on ultrastructural study on germ cell differentiations during oogenesis, the reproductive cycle and first sexual maturity of *Neptunea cumingii*.

The reproductive cycle of local population vary with the environmental factors such as water temperature and food availabity (Chung et al., 2000, 2002). Understanding the reproductive cycle and the spawning period of *N. cumingii* will provide necessary information for natural spat collections or the recruitment period and age determination of this population. In addition, data on first sexual maturity and reproductive strategy of this population are very useful information for natural resource management. Terefore, the main aim of the present study is to understand

germ cell differentiations during oogenesis, the reproductive cycle, cycle with the gonadal development and first sexual maturity of this species.

MATERIALS AND METHODS

Specimens of the neptune, *Neptunea cumingii* Crosse were collected monthly at the subtidal zone of Maldo, Kunsan, Korea, from January to December, 2002 (Fig. 1). The snails ranging from 41.0 to 106.8 mm in shell height were used for the present study. After the snails were transported alive to the laboratory, shell heights and total body weights were immediately measured.

A total of 486 individuals were used for calculation of the GSI. Monthly changes in the mean gonadosomatic index (GSI) were calculated by the following equation (Chung et al., 2002):

$$GSI = \frac{\text{The thickness of the gonad} \times 100}{\text{Diameter of posterior appendage including the gonad and digestive gland}}$$

(Fig. 2C).

Germ Cell Differentiation by Electron Microscopical Observation

For electron microscopical observations,

1) Prefixation: 2.5 % paraformal- dehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hours at 4℃.
2) Postfixation: 1 % osmium tetroxide dissolved in 0.2 M phosphate buffer solution (pH 7.4) for 1 hour at 4℃.
3) Ultrathin sections of Epon-embedded specimens were cut with glass knives with a Sorvall MT-2 microtome
4) Stain: doubly stained with uranyl acetate followed by lead citrate,
5) examined with a JEM 100 CX-2 (80 kv) electron microscope.

Gonadal Development by Histological Observations

For light microscopic examination of histological preparations, a total of 456 individuals were used for Histological analysis of the gonads and seminal vesicle tissues from January to December, 2002.

1) Fixation: Bouin's fixative for 24 h.
2) Paraffin method
3) Stain : Hansen's hematoxylin-0.5 % eosin, Mallory's triple stain and PAS stain.

First Sexual Maturity

The first sexual maturation of a total of 187 female individuals (31.4 90.5 mm in shell height) were investigated histologically in order to certify the shell heights of the snails were reached maturation and participating in reproduction from May (ripe stage) to late August (after spawning).

RESULTS AND DISCUSSION

Oogenesis, the gonadosomatic index (GSI), reproductive cycle and first sexual maturation of the female *Neptunea cumingii* have been investigated by light and electron microscope observations. In the early vitellogenic oocyte, the Golgi apparatus, vacuoles and mitochondria were involved in the formation of glycogen particle, lipid droplets and yolk granules. In the late vitellogenic oocytes, the endoplasmic reticulum and modified mitochondria were involved in the formation of proteid yolk granules in the cytoplasm near the nucleus. A mature yolk granule was composed of three components: main body(central core), superficial layer, and the limiting membrane. Monthly changes in the gonadosomatic index in females were closely associated with ovarian developmental phases. Spawning occurred between May to August, and the main spawning occurred between June and July when the seawater temperature rose to approximately 18-23℃. The female reproductive cycle with gonadal development of this species can be classified into five successive stages: early active stage (September to October), late active stage (November to February), ripe stage (February to June), partially spawned stage (May to August), and recovery stage (June to August). The rate of individuals reaching the first sexual maturity was 53.1% in females of 51.0 to 60.9 mm in shell height, and 100% in those over 71.0 mm.

REFFERENCES

FUJINAGA, K., 1987. On the growth pattern of the neptune whelk, *Neptunea arthritica* Bernardi. *Bulletin of Faculty of Fisheries Hokkaido University*, 38(3): 191-202.