

Histological and Molecular Biological Studies on Reproductive Characteristics of Hermaphroditism and Cyclic Changes in the Structures of Marsupial Gills of the Triploid Marsh clams, *Corbicula fluminea* and *C. leana*

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INTRODUCTION

The marsh clams *Corbicula* species are hermaphrodites that brood their larvae in the inner-demibranchs. Okamoto and Arimoto (1986) suggested that *Corbicula leana* reproduces by gynogenesis. *Corbicula fluminea* and *C. leana* have been reported as being triploid based on chromosomal and karyological studies. The triploid condition may be closely related to hermaphroditism. The marsh clam *C. fluminea* is one of the commercially important edible clams. However, the North American *C. fluminea* was introduced from Asia in 1900's and is now widely distributed throughout the United States where it has become a biofouling pest. There are few previous studies of the reproduction of the *Corbicula* species. In addition, gametogenesis and corresponding morphological changes in the inner-demibranchs have not been examined. Finally, there is the question as to whether reproduction of the two triploid *Corbicula* species involves self-fertilization, cross-fertilization, or parthenogenesis.

The purpose of this study was to understand functional hermaphroditism and cyclic changes in the structures of the inner-demibranchs and the duration of the pediveligers released from parent clams. Also, we compared the DNA content of spermatozoa and gill tissues of *C. fluminea*.

MATERIALS AND METHODS

The 368 specimens of *C. fluminea* and the 343 specimens of *C. leana* were collected from the Uiam Reservoir, in Korea between February 2000 and December 2001. The materials were collected monthly 30 to 40 individuals for this study. After the clams were transported alive to the laboratory of School of Marine Life Science, Kunsan National University, shell lengths and heights were measured by Vernier caliper and their total weight was determined using a balance. Histological preparations were made for study of sexuality and for study on morphological changes in the structures of the demibranchs by light microscopy. The visceral mass and the gill tissues were subjected to standard histological procedures (dehydrated in alcohol and embedded in paraffin) and sectioned at 5~7 μ m using a rotary microtome. Sections were then mounted on glass slides stained with Hansen's haematoxylin-0.5% eosin and Mallory's triple stain, and observed using the light microscope.

To compare the relative DNA content of spermatozoa and gill tissue of *C. fluminea*, cells were isolated on a glass slide by cutting a small piece of gonad and gill tissues in distilled water with a scalpel and air-drying it before fixing it with 70% ethanol. Spermatozoa and gill cells from one individual were placed on the slide. The cells were stained with the DNA-specific dye PI (propidium iodide) and DAPI (4, 6-diamidino-2-phenylindole) and the relative DNA content (fluorescence intensity) per cell was estimated by microfluorometry as in Komaru et al. (1988). DNA content assayed at least three times at each of three different concentrations of spermatozoa

and gill cells. Twelve individuals were used for this assay. Spermatozoa could be easily distinguished from other spermatogenic cells because of their elongate and curved morphology.

RESULTS AND SUMMARY

The marsh clams, *Corbicula fluminea* and *C. leana*, are functional hermaphrodite. They usually appear to be surrounded by numerous spermatozoa in the hermaphroditic follicles. In both species, the follicular ganglia (consisting of the neuronal fiber and neuronal soma-like cells at its periphery) are associated with neurosecretion and the differentiation of complex innervated nerve structures during spermatogenesis and are widely distributed in the follicles in the ripe and spawning stage. *Corbicula fluminea* and *C. leana* have two pairs of gills, with the inner-demibranchs acting mainly as marsupia. The non-marsupial demibranchs are not separated, but in the marsupial demibranchs, cyclic change in the structures of the inner-demibranchs of the gills appear, with the depletion of ripe eggs during incubatory periods and the production of mature and ripe eggs during nonincubatory periods. The reproduction of triploid *C. fluminea* and *C. leana* may occur by parthenogenesis without self-fertilization (or cross-fertilization) by eggs and sperm. The DNA contents of the somatic (gill) and gamete (spermatozoa) cells of *C. fluminea* are the same. Because reproduction is parthenogenetic, numerous spermatozoa may participate in the activation of the mature eggs and egg cleavage, as a stimulus only for parthenogenesis in the same hermaphroditic follicle or the gonophore.

REFERENCE

Okamoto, A. & B. Arimoto. 1986. Chromosome of *Corbicula japonica*, *C. sandai* and *C. (Corbiculina) leana* (Bivalvia: Corbiculidae). Venus 45:194-202.