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Gonadal Development and Reproduction in the Trumpet Shell, *Charonia sauliae*

Kyoung Ho Kang, Jae Min Kim, Seung Cheon Seon, Liang Liang Zhuo and Sang Min Lim

Division of Marine Technology, Chonnam National University, Yeosu 550-749, Korea

ABSTRACT

This study devised and tested a histological staging system for gonadal development in the trumpet shell, *Charonia sauliae*, which was collected along the south coast of Jeju Island, South Korea. This paper details for the first time the gonadal development characteristics of *C. sauliae*. Ovary and testis development in *C. sauliae* can be roughly divided into five stages: growing, mature, spent, degenerative, and resting. The trumpet shelll has multiple fecundation and fertilization during the spawning season for out-of-step gonadal development in high-temperature and low-salinity environments. Female trumpet shell tended to have larger shells and were more abundant than males (64.26% of all animals collected were female).

Key words: *Charonia sauliae*, histology, reproductive cycle.

INTRODUCTION

The trumpet shell *Charonia sauliae*, an endangered species, belongs to the family Cymatiidae and is distributed widely across tropical and semitropical areas of the Atlantic, Indian, and Pacific Oceans. Its steepled spiral shell is white with a few brown spots and can reach lengths of 10 - 30 cm. As a flesh-eating mollusk that inhabits coral reefs in seas 50-250 m deep, it plays an important role in energy transfer in coral reef communities.

There are several means of assessing the reproductive cycle in mollusks. These methods include (1) visual observation relative to the size, shape, and color of the gonads (Mason, 1958); (2) the gonadosomatic index (GSI), the ratio of gonad weight to body weight (Juhel *et al.*, 2003); (3) mean gamete diameter (Kennedy and Battle, 1964; Muranaka and Lannan, 1984); and (4) developmental staging based on certain cytological characteristics (histological techniques; Katkansky and Sparks, 1966; Yakovlev, 1977; Juhel *et al.*, 2003). Histological examination of the gonad allows direct determination of the developmental status of an individual. For example, seasonal variation in the stages of gametogenesis has been followed through histological examination (citation). Histological studies also allow identification of any phenomena likely to affect reproductive activity in bivalves (Paulet, 1990). Moreover, this technique removes the influences of other factors, especially if gonadal sex can not be distinguished macroscopically. Therefore, histological techniques are much more practical and accurate for determining gonadal development than other methods.

This work documents the ultrastructure of the gonads in both sexes of *C. sauliae*, through histological and ultrastructural analyses of different stages of development in male and female germ cells, as well as their physiological activity during the reproductive cycle. To the best of our knowledge, no related studies on the reproductive cycle of *C. sauliae* have been published. We describe and evaluate a staging system for gonadal development in *C. sauliae*, and compare the reproductive cycle of *C. sauliae* with previous studies of other species.

MATERIALS AND METHODS

Ten *C. sauliae* individuals were collected each

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Corresponding author: Kang, Kyoung Ho
Tel: +82 (61) 659-3165 e-mail: mobidic@chonnam.ac.kr
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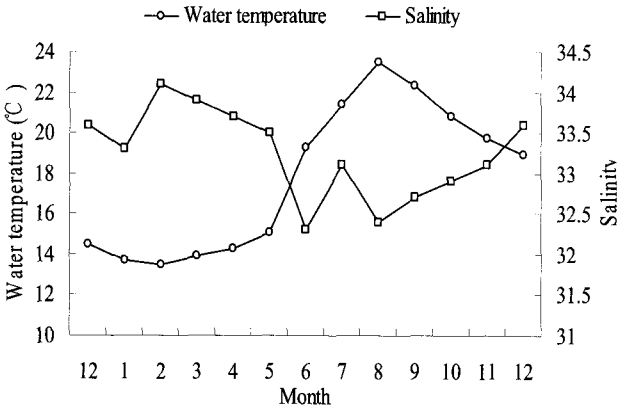

Fig. 1. Variation in water temperature and salinity at the sampling sites during the year.

month from Jeju Island, South Korea, for one year. Shell size and body weight were measured (Table 1). Individuals were used for a histological study of gonads and the stages of development of their germ cells were determined. Changes in water temperature and salinity during the sampling period were recorded every month (Fig. 1).

After collection, samples were anatomized immediately and tissues were fixed using Bouin's fluid for 48 h at 4℃ (Fig. 2). The fixed tissue was removed from the shells and part of the foot was removed to expose the internal tissues. Standard histological

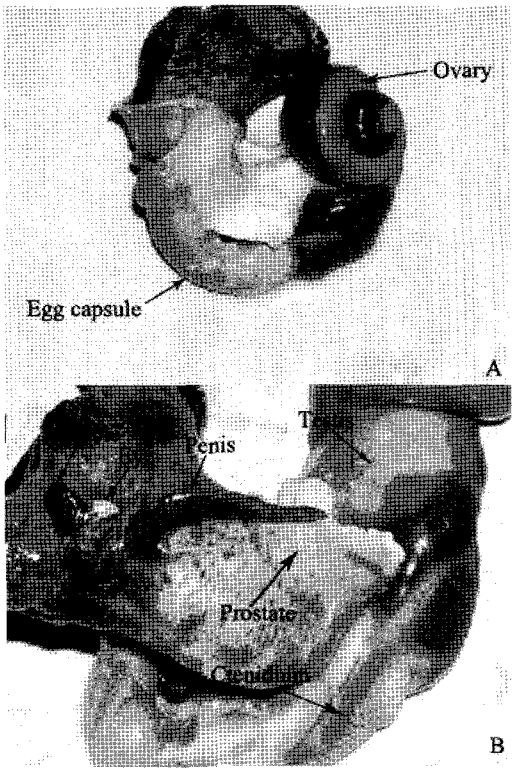

Fig. 2. Anatomy of adult trumpet shell. A: female; B: male.

methods were used. Dissected gonad tissues were then dehydrated in a graded ethanol series and embedded in paraffin, following the method described by Kiernan

Table 1. Measurements of the collected trumpet shell, *Charonia sauliae*.

Months	Shell length (cm)	Shell width (cm)	Total weight (g)	Meat weight (g)
Dec.	20.5 ± 5.2	8.1 ± 1.3	903.3 ± 124.6	617.3±48.4
Jan.	18.4 ± 3.3	7.9 ± 1.7	847.0 ± 109.5	554.3±58.2
Feb.	19.2 ± 4.0	8.0 ± 1.4	876.8 ± 140.8	580.1±49.3
Mar.	22.6 ± 5.7	10.2 ± 1.8	918.1 ± 177.2	601.7 ± 74.0
Apr.	19.4 ± 4.9	9.2 ± 2.1	802.5 ± 108.7	571.9 ± 52.2
May	21.1 ± 6.2	10.1 ± 1.3	829.7 ± 132.2	544.6 ± 47.3
Jun.	20.0 ± 5.5	8.7 ± 1.8	732.4 ± 102.0	525.4 ± 82.0
July	19.2 ± 5.3	8.4 ± 1.2	715.6 ± 149.8	503.7 ± 63.5
Aug.	18.2 ± 4.5	7.6 ± 1.1	719.8 ± 124.2	499.2 ± 45.8
Sep.	21.2 ± 5.3	10.5 ± 2.0	850.9 ± 201.5	567.3 ± 68.3
Oct.	20.5 ± 2.2	10.1 ± 1.9	893.0 ± 194.4	603.6 ± 46.1
Nov.	17.8 ± 4.7	7.0 ± 1.4	729.4 ± 107.2	523.5 ± 46.5

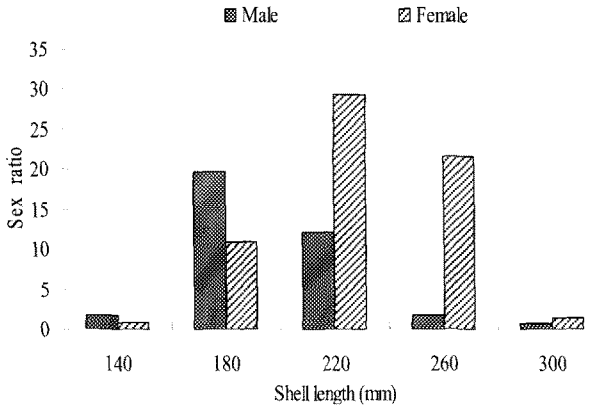

Fig. 5. Sex ratio of the trumpet shell *Charonia sauliae* based on shell length.

(1990). Standard 7-μm sections were stained with hematoxylin and eosin. The sections were examined at 40× magnification with an OLYMPUS CX41 microscope.

RESULTS

A descriptive staging technique for gametogenesis in *C. sauliae* was established after screening all tissues from individuals collected in all months (Table 2). The stage of gonadal development for male trumpet shell was assigned based on the presence or absence of male gametogenic cells within the follicles in the connective tissue. Female developmental stages were assigned based on the presence of ovums and oocytes in the gonad. In addition, various characteristics of the follicle, such as the shape and thickness of the follicular wall, were used as indicators to determine gonadal developmental stages. Several trumpet shell whose gender was difficult to determine might have been adults in a resting stage of development or adults that had been castrated by a parasite infection. Photographs of the gonadal development stages were taken in both male and female trumpet shell (Figs. 3 and 4).

The sex ratio of *C. sauliae* based on shell length is shown in Fig. 5. The overall sex ratio of the trumpet shell collected in this study was female:male 1.78:1. Individuals with shell lengths from 220-260 mm were mostly females (79.52%). In males, 88.21% had shells 180-220 mm long.

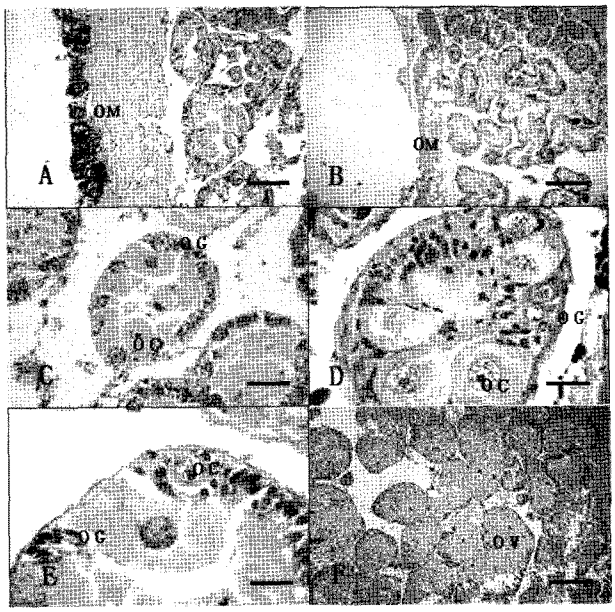

Fig. 3. The ovary of the trumpet shell at different developmental stages. A, B: growing stage; CE: mature stage; F; spent stage. OG: oogonium; OC: oocyte; OV: ovum; OM: ovarian membrane. Scale bars: A: 100 μm; B: 50 μm; C: 15 μm; D: 100 μm; E: 25 μm; F: 15 μm.

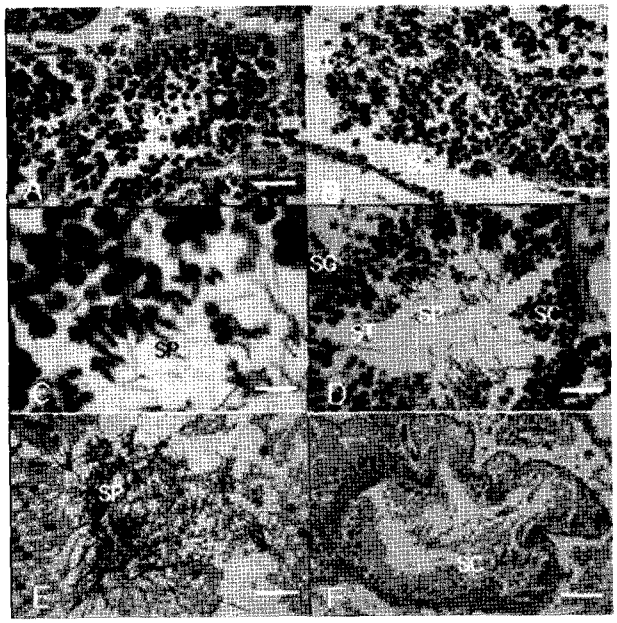

Fig. 4. The testis of the trumpet shell at different developmental stages. A: growing stage; BD: mature stage; E: spent stage; F: resting stage. SP: sperm; ST: spermatid; SC: spermatocyte; SG: spermatogonium. Scale bars: A, B, D, E: 25 μm; C: 6 μm; F: 150 μm.

DISCUSSION

In this study, a staging system for gonadal development in *C. sauliae* was devised and evaluated using tissue sections prepared using histological techniques. Histological gonadal development staging systems have been developed for a wide range of bivalve mollusks (Mann, 1979; Lango-Reynoso, 2000; Steele and Mulcahy, 2001; Juhel *et al.*, 2003).

This study outlines the histological aspects of trumpet shell gonads and allows more precise determination of gonadal development (Table 1). To date, this is the first staging system developed for *C. sauliae* based on purely histological techniques.

By comparing tissue sections at different developmental stages, we found that ovum developmental stage varied both within and among follicles. In ovary sections of a female individual that just had spawned, some follicles remained plump and were rich in ovums and oocytes that could have spawned after a short period of development. This pattern has been observed previously in *Spisula solidissima* (Lin *et al.*, 2005). This observation could explain why the trumpet shell has multiple fecundation and fertilization that continue for two days during the spawning season.

Previous results showed that the spawning season of *C. sauliae* ranges from December to March (citation). A number of external factors induce spawning in marine mollusks, such as water temperature, salinity, wave action, and food availability (Brethes *et al.*, 1994; Juhel *et al.*, 2003). Based on the changes in water temperature and salinity at our sampling site, environments with higher salinity and lower water temperature are appropriate spawning sites for *C. sauliae*. Rao (1973) found that temperature and salinity had no marked influence on spawning induction in *Cellana radiata*. However, the absence of spawning behavior during

Table 2. Descriptions of the gonadal development stages of male and female trumpet shell, *Charonia sauliae*.

Stage No.	Stages	Description of testis development	Description of ovary development
1	Resting stage	A few or unidentified gametes are present in this early stage of development. The whole follicular cavity collapsed significantly coupled with a lot of drapes.	Ovaries are slack and empty and have a large central lumen and a discontinuous germinal epithelium. Connective tissue is present in the gonads.
2	Growing stage	Follicular cavity becomes plump. A thick germinal epithelium lines the edges of the testis. Some germinal cells can be observed in the centre of follicular cavity.	Follicles are small and contain a small quantity of oogonia lining the edges. The oogonium was small and irregular with bulky sphere -shaped nucleus.
3	Mature stage	The follicular cavity was filled with various types of cells including spermatogoniums spermatocytes, spermatids and sperms. Mature cells trend to present at the centre.	Ovaries are swollen and there are many oocytes present. A few ovum also could be observed. The follicle layer became thinner and no longer active.
4	Spent stage	The number of sperm decline gradually with a small quantity of mature sperm in the centre of follicles. Larger gaps appeared in follicular cavities with sperm emissions.	Ovaries are showing the signs of becoming slack. Ovums present in ovaries in mass and some observed outside ovaries are in connective tissue. Some cavities with different sizes appeared.
5	Degenerative stage	Gonadal color become obviously bleak. Many haemocytes present in the tubules and outside generally surrounding residual spermatozoa.	Ovaries are slack and are showing signs of tissue destruction. Lots of degenerative ovums present in the follicles.

the months with high temperature or relatively low salinity (Rao, 1973) is consistent with our results, although the specific relationship between the environment and spawning activities of the trumpet shelll need further study.

The mean shell size of females was larger than that of males. Previous studies of marine gastropods have shown that males often die after fecundation.

Further, the larger shells of males correspond to a higher mortality rate, which implies that life expectancy should be shorter in males than in females (Rocha-Barreira, 2002). In this study, the overall sex ratio of the specimens collected throughout the year was female-biased. Overall, females represented 64.26% of individuals collected. Cardenas (2005) also found a high ratio of females to males in the fighting conch *Strombus pugilis*.

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