Gonadal Development and Reproductive Cycle of Sea Hare *Aplysia kurodai* in Jeju Coastal Waters

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ABSTRACT : Gonadal development and reproductive cycle of *Aplysia kurodai* inhabiting the coastal waters of Jeju Island, Korea were investigated based on monthly changes of gonadosomatic index, gametogenesis, and developmental phases of ovotestis. *A. kurodai* was simultaneous hermaphrodite; the ovotestis generally embedded in the posterior dorsal surface of the brownish digestive gland. The ovotestis is composed of a large number of follicles, and both oocytes and sperm are produced in the same follicles. In the sampling periods, the adult *A. kurodai* population have characteristic of seasonal pattern present during only 10 months. The reproductive cycle can be grouped into the following successive stages in the ovary: inactive (December to February), active (December to April), mature and spawning (April to September). The gonadal development of *A. kurodai* coincided with rising temperature, and spawning occurred from April to September, when the temperature was high. The histological observations of the ovotestis suggested that this species have a single spawning season that extend over six months.

Key words : Reproductive cycle, Gonadosomatic index, *Aplysia kurodai*, Ovotestis, Simultaneous hermaphrodite

INTRODUCTION

Sea hares of the species belonging to the genus *Aplysia* are benthic herbivores residing in the intertidal and subtidal zones throughout the world (Beeman, 1968; Klussmann-Kolb, 2004). They are widely used as model organism for neurobiological and behavioral studies (Kandel, 1979). Their biological and ecological characteristics are short lives, rapid growth, high egg production, and high abundance (Gev et al., 1984; Carefoot, 1987). Many studies have concentrated upon seasonal variation in weight or size (Carefoot, 1967a; Usuki, 1970; Audesirk, 1979), reproductive activity (Yusa, 1996), seasonal change in population (Plaut et al., 1998), and recruitment into the population by recently metamorphosed juveniles (Sarver, 1979). The aplysiids have a distinct seasonal occurrence and are a seasonal breeder. For example, *A. oculifera* in the northern Gulf of Eilat (Aqaba), Red Sea, has a seasonal life cycle that adult populations occur from December to July but are absent from August to November. Such seasonality of *Aplysia* spp. has also been reported among other *Aplysia* species from different localities (Carefoot, 1967b; Usuki, 1970; Lederhendler et al., 1975; Audesirk, 1979; Sarver, 1979; Gev et al., 1984; Achituv & Suswein, 1985; Pennings, 1991; Strenth & Blankenship, 1991; Plaut, 1993). Spawning period differs according to the geographical location, and spawning event showed species specificity (Usuki, 1970). The reproductive activity of aplysiids is controlled by neuroendocrine regulation (Kupfermann, 1967; Strumwasser et al., 1969; Pinsker & Dudek, 1977) and environmental factor, water temperature (Pinsker & Parsons, 1985; Wayne & Block, 1992; Wayne et al., 1996). Thus, initial investigations of the reproductive cycle of *Aplysia* spp. were motivated from the standpoint of its neurohormonal control.

As described above, the reproductive strategies of *Aplysia* spp. vary by species, and due to the variations in their eco-

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logical distribution, their morphological and biological characteristics are different. This study investigated monthly changes of gonadosomatic index, gametogenesis, and developmental phases of ovotestis in order to characterize the gonadal development and reproductive cycle of *A. kurodai* inhabiting the coastal waters of Jeju Island, Korea.

MATERIALS AND METHODS

A. kurodai were sampled by scuba diving at a depth of 3-9 meters in the coastal waters of Hamdeok, northeast of Jeju Island, Korea (33˚29' N, 126˚26' E). Samples were collected monthly from December 2002 to January 2004, except the period from October 2003 to January 2004. The day length and water temperature data were provided by KASI (Korea Astronomy and Space Science Institute) and NFRDI (National Fisheries Research and Development Institute), respectively. For histological analysis on the ovotestis development, each animal was anaesthetized in 10% $MgCl_2$ (Sigma), thereafter, pieces of the ovotestis were fixed in Bouin's solution and then embedded in paraffin. Paraffin-embedded ovotestis tissues were sectioned at 5-6 ㎛ and stained with Azan and Hansen's hematoxylin and 0.5% eosin. The specimens were examined under a light microscope. The developmental stage of ovotestis was classified as following; inactive, active, and mature and spawning stage (Table 1). The gonadosomatic index (GSI) was calculated for each individual, by using the following equation: GSI= (ovotestis weight) / (body weight) × 100. All data of GSI were expressed as mean ± SEM (standard error of mean) and were analyzed by one-way ANOVA using the computer package SYSAT (SPSS, IL USA). Tukey's multiple comparison test was performed to analyze the variance of the data among monthly GSI.

RESULTS

1. Morphological Feature and Structure of Ovotestis

A. kurodai have a single unpaired gonad, the ovotestis. In mature specimens the ovotestis was the largest of the reproductive organs and was orange-yellow in color. It was generally embedded in the posterior dorsal surface of the brownish digestive gland (Fig. 1A and B). The ovotestis was composed of a large number of follicles and the follicles are surrounded by a basement membrane. Each of follicles opens into a division of the small hermaphroditic duct (Fig. 1C). Both oocytes and spermatozoa were developed in the same follicles, and oocytes were mainly observed membrane of the follicle and numerous spermatozoa were observed the lumen of follicle (Fig. 1D). When oocytes and spermatozoa were matured, they gather into a division of the small hermaphroditic duct (Fig. 1E).

2. Monthly Change of Gonadosomatic Index (GSI)

The water temperature reached a minimum from December to February and a maximum in July to September. The GSI was lower between December and February and ranged 0.14±0.06 to 0.23±0.03. Subsequently, the GSI began to increase and reached 1.37±0.30 in April. The GSI then slightly decreased and it increased again, reached a maximum (2.12±0.11) in July. Then, GSI decreased from August to September (Fig. 2).

Table 1. Reproductive stages in ovotestis development of *Aplysia kurodai*

Classification	Histological criteria
Inactive stage	Follicles are emptied and no evidence of gonadal development
Active stage	Follicles were contained the spermatocytes, spermatids and spermatozoa but only unyolked and early growing oocytes
Mature and spawning stage	Mature oocytes occupied the majority of the follicles and numerous sperm within a division of the small hermaphroditic duct

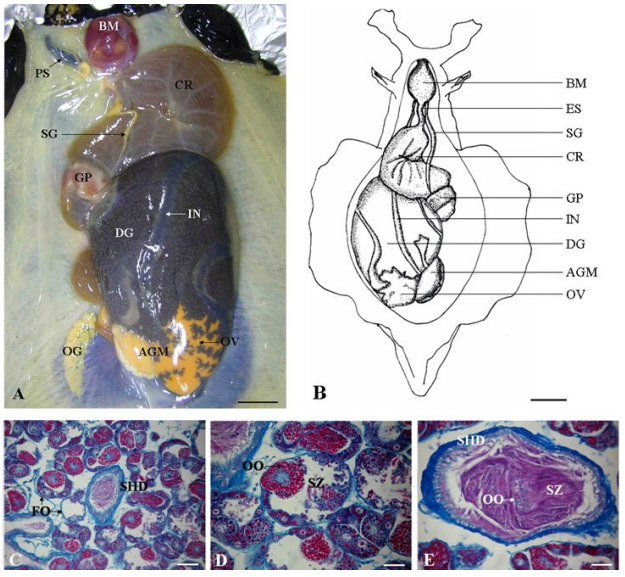

Fig. 1. Anatomical features and histological sections ovotestis stained with Azan of *Aplysia kurodai*. A, anatomical feature of ovotestis and digestive system. B, schematic outline of ovotestis and digestive system. C, ovotestis of composing a large number of follicles. D, oocytes and spermatozoa of developing in the same follicles. E, oocytes and spermatozoa of collecting in small hermaphroditic duct. AG, anterior gizzard; AGM, accessory genital mass; AN, anus; BM, buccal mass; CR, crop; Dg, digestive gland; ES, esophagus; FO, follicle; GA, ganglia; GP, grinding plates; IN, intestine; OG, opaline gland; OO, oocytes; OV, ovotestis; PG, posterior gizzard; PS, penis; SG, salivary gland. SHD, small hermaphroditic duct; SZ, spermatozoa. Scale bars indicate 1.0 ㎝ (A and B) and 50 ㎛ (C to E).

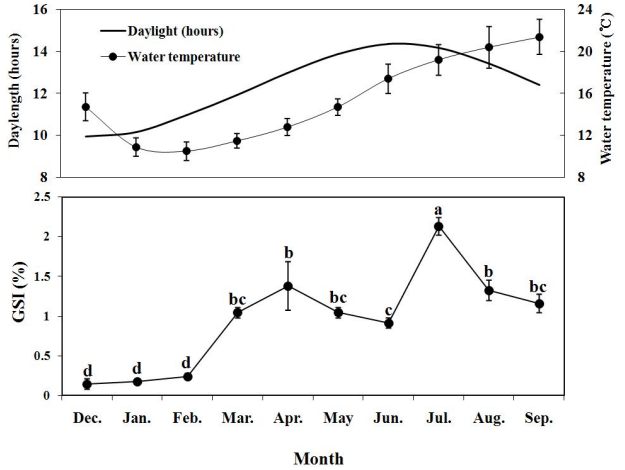

Fig. 2. Monthly changes in water temperature, day length and gonadosomatic index (GSI) of *Aplysia kurodai* in the coastal waters of Hamdeok, northeast of Jeju Island from December 2002 to September 2003. Different letters denote significant difference between month (P<0.05). Vertical bars represent the standard error.

3. Gemetogenesis

The ovotestis was composed of a large number of follicles, and the follicles were surrounded by basement membrane. Both oocytes and sperm were produced in the same follicles. The unyolked oocytes were approximately 5.0-7.5 ㎛ in diameter, and had a small cytoplasmic volume, since the 4.0-6.0 ㎛ nucleuses occupied the majority of the intracellular space. Unyolked oocytes were mainly observed in the basement membrane of follicle (Fig. 3A). Unyolked oocytes accumulated uniform granular material in the cytoplasm and attained a diameter of approximately 15.0 ㎛, containing a voluminous nucleus with a nucleolus (Fig. 3B). The early growing oocytes were approximately 30.0-40.0 ㎛ in diameter, and their cytoplasm began to accumulate yolk granules (Fig. 3C). The mature oocytes were approximately 62.5-75.0 ㎛ in diameter, with numerous yolk granules

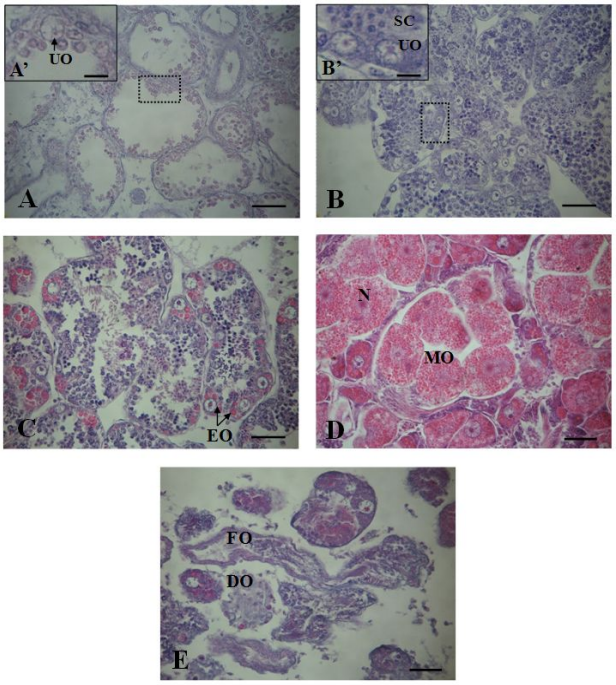

Fig. 3. Oogeneis of *Aplysia kurodai*. A and B, unyolked oocytes; C, early growing oocytes; D, mature oocytes; E, degeneration undischarged oocytes. DO, degenerative oocyte; N, nucleus; MO, mature oocyte; FO, follicle; UO, unyolked oocyte. Scale bars indicate 50 ㎛. The inset of A and B show unyolked oocytes in basement of follicle. Scale bars denote 5-㎛ length.

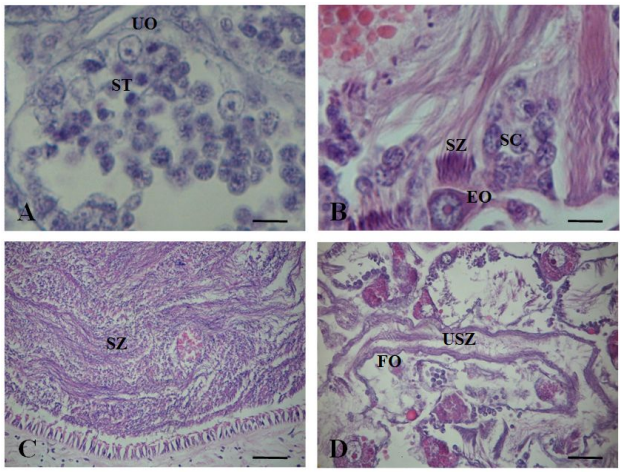

Fig. 4. Spermatogeneis of *Aplysia kurodai*. A, spermatocytes and spermatids clustered around unyolked oocytes. B, sperm bundles in basement follicle. C, numerous spermatozoa within a division of the small hermaphroditic. D, degeneration of undischarged spermatozoa. FO, follicle; SC, spermatocyte; SHD, small hermaphroditic duct; ST, spermatid; SZ, spermatozoa; UO, unyolked oocyte; USZ, undischared spermatozoa; YG, young oocyte. Scale bars denote 10-㎛ (A and B) and 50-㎛ (C and D) length.

homogeneously distributed in the cytoplasm (Fig. 3D). Thereafter mature oocytes were released from the ovotestis via small hermaphroditic duct, and the undischarged oocytes degenerated and follicles were constricted (Fig. 3E). The spermatocytes and spermatids clustered around unyolked oocytes or inside the basement membrane of follicle (Fig. 4A). As the ovotestis developed, numerous spermatozoa occupied the lumen of follicle (Fig. 4B). The mature sperm were collected into a division of the small hermaphroditic duct and was stored prior to copulation (Fig. 4C). Thereafter, the undischarged spermatozoa degenerate, and the follicles were constricted (Fig. 4D).

4. Developmental Phases of Ovotestis

The developmental phases of ovotestis of *A. kurodai* were divided into following three stages (Fig. 5 and 6).

1) Inactive Stage

The ovotestis of inactive stage had a few unyolked oocytes with about 5.0-7.5 ㎛ in diameter and sperm bundles in follicle, but most of the follicle emptied (Fig. 5A). This stage was observed from December to February (Fig. 6).

2) Active Stage

The ovotestis of active stage had unyolked oocytes and a few early growing oocytes. The early growing oocytes were about 20.0-30.0 ㎛ in diameter and began to accumulate yolk granules in their cytoplasm. Also, the follicle

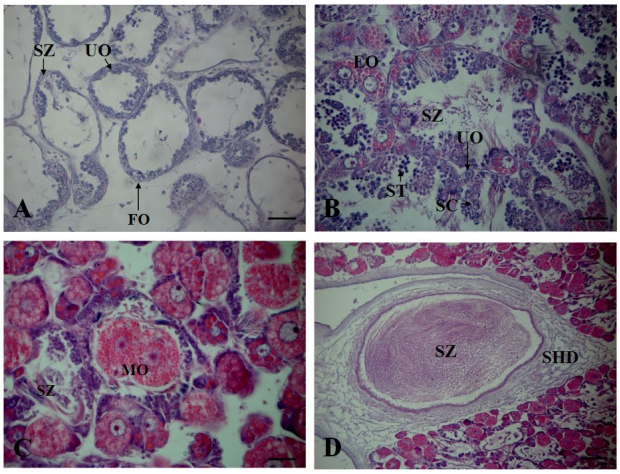

Fig. 5. Histological sections of ovotestis at different gonadal stages of *Aplysia kurodai*. A, inactive stage; B, active stage; C, mature and spawning stage, D, numerous sperm within a division of the small hermaphroditic duct. EO, early growing oocyte; FO, follicle; MO, mature oocyte; SHD, small hermaphroditic duct; SC, spermatocyte; ST, spermatid; SZ, spermatozoa; UO, unyolked oocyte. Scale bars denote 40-㎛ (A to C) and 100-㎛ (D) length.

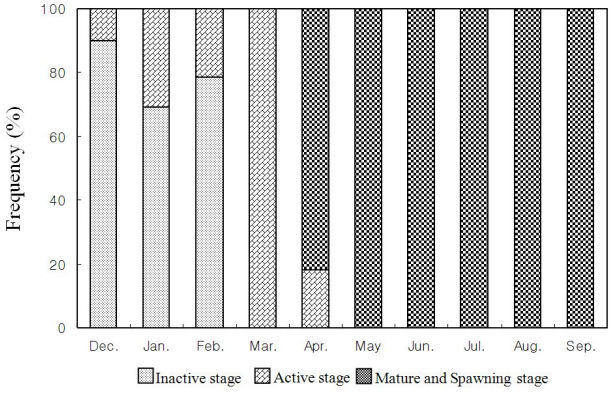

Fig. 6. Frequency of ovotestis developmental phase of *Aplysia kurodai* in the coastal waters of Hamdeok, northeast of Jeju Island from December 2002 to September 2003.

was observed spermatocytes, spermatids and sperm bundles (Fig. 5B). This stage was observed from December to March (Fig. 6).

3) Mature and Spawning Stage

The ovotestis of mature and spawning stage had a few early growing oocytes but mature oocytes occupied the majority of the lumen. The mature oocytes were approximately 62.5-75.0 ㎛ in diameter with numerous yolk granules that were homogeneously distributed in the cytoplasm. The follicles also contained spermatocytes, spermatids and sperm bundles (Fig. 5C). Particularly a numerous sperms were observed within a division of the small hermaphroditic duct (Fig. 5D). This stage was observed from April to September (Fig. 6).

DISCUSSION

Opisthobranchs are simultaneous hermaphrodite, i.e., adult animals having a functional female as well as male reproductive system, and lay egg masses by internal cross-fertilization after copulation (Kandel, 1979; Beeman, 1970; Blankenship et al., 1983; Switzer-Dunlap et al., 1984). The most of opisthobranchs have a single unpaired gonad, the ovotestis, which is composed of large number follicles. In many opisthobranchs, oocytes and sperm are produced in the same follicles, however in only a few species, complete separate male and female follicle are found (Reid, 1964), and separate male and female gonads are also found in some Acochlidiacea (Morse, 1976). The structure and developmental tendencies of ovotestis in *A. kurodai* were similar to those previously reported for other *Aplysia* spp. (Beeman, 1970; Dudek et al., 1980). In this study, *A. kurodai* had a single unpaired gonad, the ovotestis, which was composed with numerous follicles. The follicles either partially or completely embedded in the digestive gland tissue, from which they were separated by the basal lamina. Each follicle contained a mixture of both male and female germ cells in different stage after the onset of sexual maturation. However, sperm maturation just preceded oocytes maturation. These results suggested that the spawning of *A. kurodai* does not always coincide with copulation, and inconsistency of oocytes and sperm development in *A. kurodai* is specific reproductive strategy.

The aplysiids are a seasonal breeder and the reproductive activity is controlled by neuroendocrine regulation (Kupfermann, 1967; Strumwasser et al., 1969; Pinsker & Dudek, 1977) and environmental factor, water temperature (Pinsker & Parsons 1985; Wayne & Block, 1992; Wayne et al., 1996). Their reproductive season was also related to the geographic distribution of the species and its specific reproductive strategy (Usuki, 1970). Most of the previous studies on the reproduction of *Aplysia* spp. have reported the periodicity of occurrence and high variability in abundance and population structure at different sites (Usuki, 1970; Susswein, 1987; Pennings, 1991; Strenth & Blankenship, 1991). Particularly, the adult *Aplysia* population has characterized that seasonal pattern present during only 5-6 months every year and this pattern is related with abundance of food such as algae (Usuki, 1970; Audesirk, 1979; Sarver, 1979; Gev et al., 1984; Susswein et al., 1987; Strenth & Blankenship, 1991). For instance, adult *A. oculifera* inhabit the intertidal and subtidal zones for 5 months every year, during winter and spring, when the green algae Enteromorpha intestinalis and *Ulva* spp. are abundant (Susswein et al., 1987; Plaut, 1993; Plaut et al., 1998). Also, the period of spawning of *Aplysia* spp. is relatively long; for instance, spawning of *A. californica* in southern California occurs between late spring and early fall with a peak in summer, and the increasing water temperature in spring provides a synchronizing cue for the initiation of gonadal development (Audesirk, 1979). Usuki (1970) suggested that *A. kurodai*, *A. parvula* and *A. juliana* found in the Sado of the Japan had almost similar spawning patterns, two distinct spawning periods in a year, such as from April to July and from November to December or January. However, the spawning period in each species was determined based on discovery of the egg masses in the field or aquaria. In this study, the

gonadal development of *A. kurodai* coincided with rising water temperature, and spawning occurred from April to September, when the temperature was high. The histological observations of the ovotestis suggested that this species have a single spawning season that extend over six months.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea Grand funded by the Korean Government [NRF-2009-353-F00026].

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(Received 2 September 2011, Received in revised form 19 September 2011, Accepted 21 September 2011)