

SEASONAL CHANGES IN REPRODUCTION AND BIOCHEMICAL COMPOSITION OF THE COCKLE, *FULVIA MUTICA* REEVE (1884), IN CHEONSU BAY OFF THE WEST COAST OF KOREA

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ABSTRACT This is the first study of seasonal changes in gonad development and tissue biochemical composition of the cockle *Fulvia mutica* in Cheonsu Bay off the west coast of Korea. Gametogenesis commenced in December when the surface water temperature was 9.1°C. In May, the first discharge of oocytes was observed, and spawning continued until October. The condition index (CI), a ratio of tissue dry weight to shell dry weight, increased gradually from late May to July and declined dramatically in August and September, indicating that active discharge of gametes occurred in the bay during this period. Histology and the CI suggested that *F. mutica* spawning in Cheonsu Bay is continuous from early summer to early fall, with two spawning peaks in June and July, and September and October. All cockles examined in November were sexually inactive. The relatively low CI recorded in late fall to mid winter (November to January) coincided with high levels of ash and low levels of lipids and carbohydrates in the tissue, whereas protein levels remained unchanged. The observed spawning pattern of *F. mutica* in Cheonsu Bay suggests that the currently established timing for fishing closure, from July to September, should be expanded from June to October to protect early-spawning broodstocks. This change may secure recruitment and lead to a subsequent increase of the wild cockle population in the bay.

KEY WORDS: *Fulvia mutica*, cockle, gametogenesis, biochemical composition, broodstock management, Korea

INTRODUCTION

The cockle, *Fulvia mutica* (Reeve 1844) is a hermaphroditic cockle commonly occurring in the sand-mud lower intertidal zone to depths of 10–30 m along the west and south coasts of Korea (Min 2004), and along the coastal Yellow Sea in China (Qi 2004, Liu et al. 2008a, Liu et al. 2008b) and Japan (Nogami et al. 1981, Ganmanee et al. 2004). The cockle is a highly priced shellfish in Korea, and annual landings in Korea rely entirely on its wild fishery. In 1993, 11,226 metric tonnes (MT) cockles were reported to be landed in Korea, but landings dropped dramatically to 428 MT in 1995. For the past decade, cockle landings have fluctuated between 1,000 MT and 3,000 MT annually, which is much reduced from the landing levels recorded before 1993. To date, no clear explanation for this striking decrease in cockle landings in Korea has been achieved, although water quality changes and overexploitation are believed to be responsible for the decline (Park et al. 2006a). Several studies have reported that population decreases in marine bivalves in coastal regions are often associated with overfishing, environmental pollution, and disease (Peterson 2002, Gangloff et al. 2008, Ren et al. 2008). To protect the spawning broodstock and to promote the subsequent enhancement of the wild population, fishing is often suspended for a certain period based on the annual gametogenesis of the species. On Jeju Island, off the south coast of Korea, the top shell fishery of *Batillus cornutus* is closed from July to September, because the species is actively engaged in spawning during this period (Cha et al. 2004).

Annual reproductive cycles of iteroparous marine bivalves in temperate environments are characterized by several gametogenic stages, including a vegetative phase when energy reserves are actively stored, cellular differentiation, proliferation of the

germ cells, release of ripe eggs and sperm during spawning, and recovery and rest (Giese & Kanatani 1987, Gosling 2003, Saucedo & Southgate 2008). Such reproductive processes are directed internally by stored reserves and endocrine chemicals, and externally by water temperature, salinity, and food availability in the water column (Sastry 1975). Recent studies of marine bivalve reproduction have demonstrated that sex steroids such as estradiol-17 beta and testosterone are involved in vitellogenesis and subsequent spawning (Gauthier-Clerc et al. 2006, Wang & Croll 2006, Liu et al. 2008a).

Located off the west coast of Korea, Cheonsu Bay (36°40'–36° 70' N, 126°35'–126°50' E) is a semiclosed bay where a cockle fishing ground has been established (Fig. 1). In 2005, the annual landing of cockles in this bay was recorded as 29 MT, with annual landings ranging from 0–1.156 MT between 1995 and 2005. Such wide variation in the landings in this bay is yet to be understood, and overfishing is considered to be one of the main factors responsible for the decrease (Park et al. 2006b). Currently, the *F. mutica* fishery by trawl in the bay is closed from July to September to protect spawning individuals, although the annual gametogenesis and reproductive physiology of the cockle in this area has not been thoroughly elucidated. In an attempt to clarify the annual gametogenesis of *F. mutica* in Cheonsu Bay, we first investigated annual gametogenesis and seasonal changes in biochemical composition using histology. Based on the observed annual reproductive cycle, we reevaluated the currently established period of fishing closure for *F. mutica* in Cheonsu Bay.

MATERIALS AND METHODS

Sampling Effort

Thirty adult cockles ranging from 60–90 mm in shell length (SL; the longest axis of the shell) were collected each month

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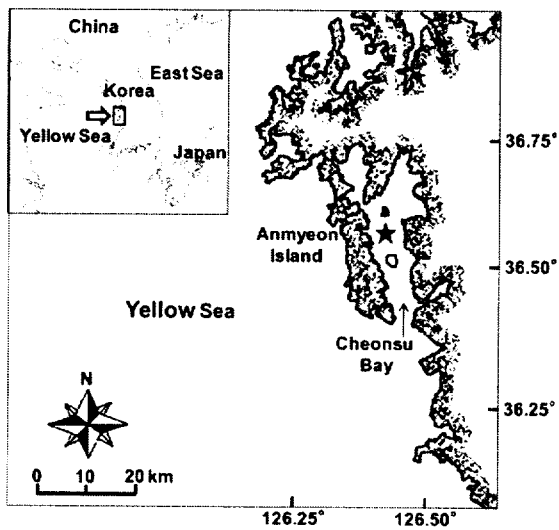


Figure 1. Location of the sampling area.

from March 2005 to February 2006 using a dredge net. After recording SL, the soft body was removed and weighed to 0.1 g. Shell weight was also recorded after the shells had dried completely. Table 1 summarizes the SL and tissue wet weight of cockles used in this study. For the histological examination of gonads, a 2–3-mm-thick transverse section was made in the middle of the foot (Fig. 2). Residual soft tissues were then freeze-dried, and the dry tissue weight (DTW) was measured. Last, lyophilized tissues were homogenized using a mortar to measure the protein, carbohydrate, and lipid levels of the tissues.

Histology

The transverse sections were fixed in Davidson's solution for 48 h. After being dehydrated in alcohol and embedded in paraffin, the block was sliced to 5 μ m and stained with Harris hematoxylin and eosin Y. The reproductive maturities of the testis and ovary prepared in the histological slide were examined under a light microscope and categorized into 1 of 6 stages (early development, late development, ripe, spawning, spent,

TABLE 1.

The size and number of *F. mutica* analyzed in the current study.

Period	n	Shell Length (mm)	Tissue Wet Weight (g)
March 2005	30	76.4 \pm 4.1	51.90 \pm 11.71
April	30	78.9 \pm 4.4	60.01 \pm 10.83
May	30	75.6 \pm 3.6	51.86 \pm 7.95
June	30	77.9 \pm 4.2	56.52 \pm 7.64
July	30	77.8 \pm 4.0	57.14 \pm 10.02
August	30	72.8 \pm 7.2	40.77 \pm 13.01
September	30	78.8 \pm 4.5	46.53 \pm 8.66
October	30	81.0 \pm 5.3	49.52 \pm 12.68
November	30	62.7 \pm 2.1	16.57 \pm 2.81
December	30	72.6 \pm 4.0	35.36 \pm 7.34
Jan 2006	30	78.0 \pm 6.1	39.62 \pm 9.67
February	30	64.5 \pm 3.2	27.17 \pm 4.81

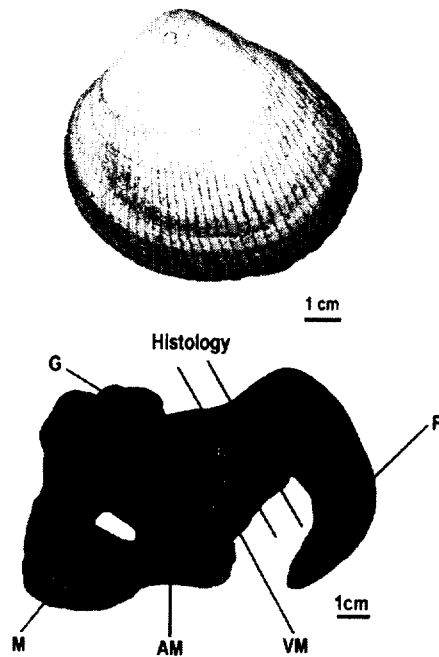


Figure 2. Photograph of *F. mutica* soft tissue. AM, adductor muscle; F, foot; G, gill; M, mantle; VM, visceral mass.

and resting) according to Kang et al. (2003). Oocyte diameters were also measured from digitized images of the ovaries using image analyzing software.

Biochemical Composition of Tissue

Total protein levels in tissue were determined using a BCA protein assay kit (Pierce, Rockford, IL). After hydrolyzing 20 mg lyophilized tissue with 0.1 M NaOH, the optical density of the supernatant was measured at 562 nm with a spectrophotometer, and the protein level in the supernatant was inferred from the bovine serum albumin prepared as a standard in the assay (Lowry et al. 1951, Navarro et al. 1989). Total carbohydrates were measured using the phenol-sulfuric acid method described by Taylor (1955). Dextrose (anhydrous) was used as the standard in the assay. Total lipids in the tissue were then measured gravimetrically after extracting lipids from the tissue with methanol and chloroform according to Bligh and Dyer (1959). The ash content of the tissue also was estimated gravimetrically by combusting a known amount of the lyophilized tissue at 500°C for 12 h.

Condition Index

The condition index (CI) was calculated as the ratio of DTW to the shell dry weight (SDW):

$$CI = (DTW/SDW) \times 100$$

Water Temperature and Salinity

Seasonal changes in the water temperature and salinity in the bay during the study period were obtained from the National Oceanographic Research Institute and the Marine Environmental Information Service Center of Korea. As shown in Figure 3,

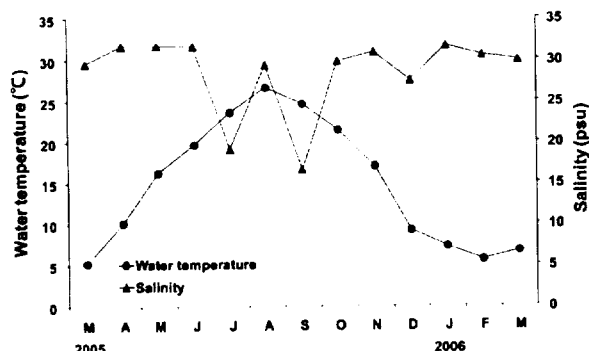


Figure 3. Seawater temperature and salinity variation in Cheonsu Bay from March 2005 to February 2006.

the temperature and salinity ranged from 5.2–26.5°C and 16.5–31.7 psu, respectively, from March 2005 to February 2006.

RESULTS

Annual Reproductive Cycle

Figure 4 plots the monthly changes in gametogenesis observed in ovaries and testes. Resting cockles could be observed from September to January. In November, when the water temperature remained at 17°C, all cockles examined were in the resting phase, exhibiting no testis or ovary. Oogenesis and spermatogenesis began as early as December (water temperature, 9°C), when 52% and 32% of ovaries and testes, respectively, were in the early developing stage. As the water temperature increased rapidly from March (5°C) to May (16°C) in 2005, the proportion of late developing ovaries and testes also increased dramatically

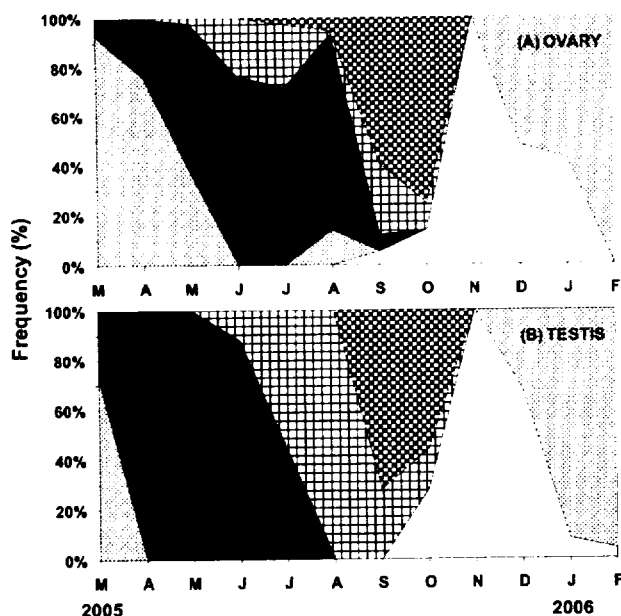


Figure 4. (A, B) Frequency distribution of gametogenic stages in the ovaries (A) and testes (B). Six gametogenic stages were categorized: resting (□), early development (▨), late development (■), ripe (▩), spawning (▧) and spent (▦).

from 7.3–50.0% and 28.0–80.0%, respectively. In May, 10.8% of cockles were in the ripe stage and were ready for spawning, and the first spawning cockles (2.3%) were observed in this month. The proportion of ovaries in the spawning stage increased in June (23.6%) and July (25.5%), indicating that massive spawning of *F. mutica* occurred during this period, when water temperatures ranged from 20–24°C. In August, the proportion of ovaries in the spawning stage reduced dramatically to 1.3%, although the proportion of ovaries in the late development and mature stages still remained high (40.0% and 39.3%, respectively). In September, the proportion of ovaries in the spawning phase increased again to 28.7%, before dropping to 10.7% in October. In October, most cockles (75.3%) exhibited spent ovaries. The annual reproductive cycle of cockles in the bay could be summarized as sexually indifferent (i.e., resting) from September to February, early developing from December to April, late developing from March to July, spawning from June to September, and spent from September to October.

Figure 5 shows monthly changes in oocyte sizes. The monthly mean oocyte size varied from 7.6 (in the early developing stage in March)–98.4 μm (in the ripe and ready for spawning stage in June). Small oocytes, 10–30 μm in diameter, were dominant from December to February, when early developing oocytes were growing in the follicles. In spring, the oocyte size increased from 20–40 μm in March to 50–60 μm in May. From June to August, when cockles were spawning, the oocyte size remained at 50–60 μm .

Tissue Biochemical Composition

The total carbohydrate content of the tissue ranged from 61.4 (October)–342.2 mg/g DTW (June, Fig. 6). A rapid decline in total carbohydrate level was observed from June to August, coinciding with ovary maturation and subsequent spawning during this period. In November, carbohydrate levels were recorded at their annual minimum. Carbohydrate levels rose exponentially from January (85.5 mg/g DTW) to February (244.0 mg/g DTW). The total protein in tissue varied annually from 276.1 (April)–384.6 mg/g DTW (September). Compared with the total carbohydrate levels, total protein levels were fairly stable throughout the year, and no clear seasonality was observed. Total lipid levels in the tissue varied from 61.8 (November)–123.7 mg/g DTW (February). The highest ash level in the tissue was recorded in November (226.2 mg/g DTW), and the level declined rapidly from January (206.4 mg/g DTW) to February (134.4 mg/g DTW). Monthly changes in ash content were negatively correlated with total carbohydrates (Pearson's correlation coefficient, $r = -0.789$, $P < 0.001$), total lipids ($r = -0.619$, $P < 0.001$), and CI ($r = -0.444$, $P < 0.001$).

Condition Index

CI, the ratio of tissue dry weight to shell weight, varied from 25.0 in January to 36.2 in July (Fig. 7). From May to July, CI increased gradually as the gonads became ripe and ready for spawning. CI dropped dramatically from July (36.2) to September (26.6), and the decline coincided with the spawning of cockles during this period. CI increased again from November (26.2) to December (32.1), then dropped again in January (25.0). Histology indicated that the cockles analyzed during this period were mostly in the resting or early developing stages, indicating that the increases and decreases in CI were not associated with

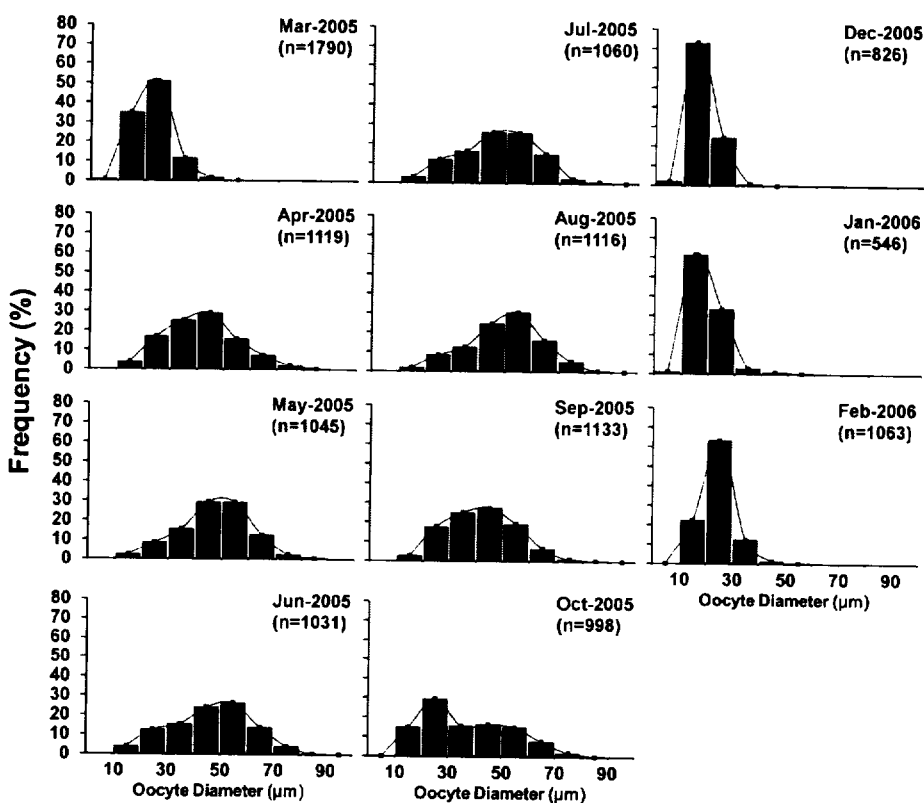


Figure 5. Frequency distribution of the oocyte diameter size classes.

spawning activity. CI increased noticeably from January (25.0) to February (32.6) when the total lipid and carbohydrate levels in the tissue also increased dramatically (Fig. 6).

DISCUSSION

Few studies have reported on the annual reproductive cycles of *F. mutica* in Korean waters. Chang and Lee (1982) investigated

the annual reproductive cycle of *F. mutica* in Gamakman Bay off the south coast of Korea, 230 km to the south of the current study site. In Gamakman Bay, cockles initiated gametogenesis in October, when the water temperature reached 20°C, and spawning cockles could be observed as early as May, when the water temperature remained at 17°C. Chang and Lee (1982) reported a spawning period for the cockle in Gamakman Bay from May to October, with 2 spawning peaks: one in June to August and the

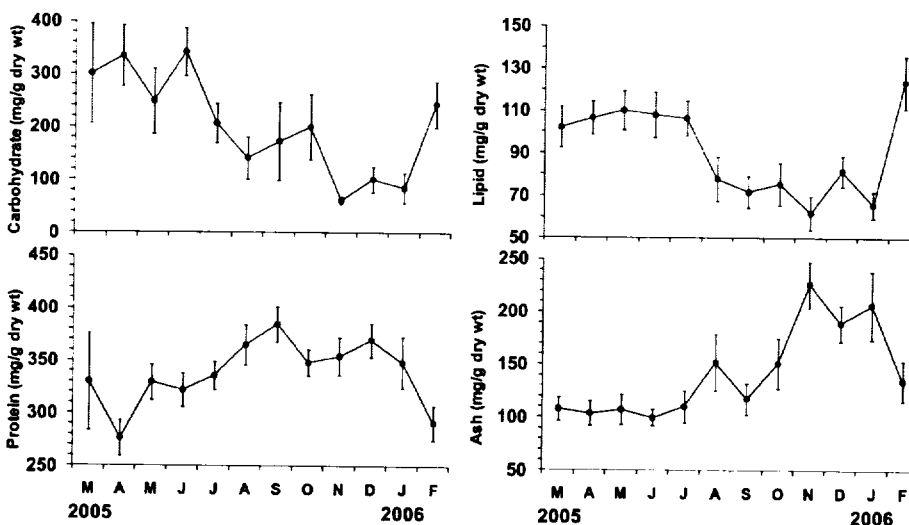


Figure 6. Seasonal changes in the tissue biochemical composition.

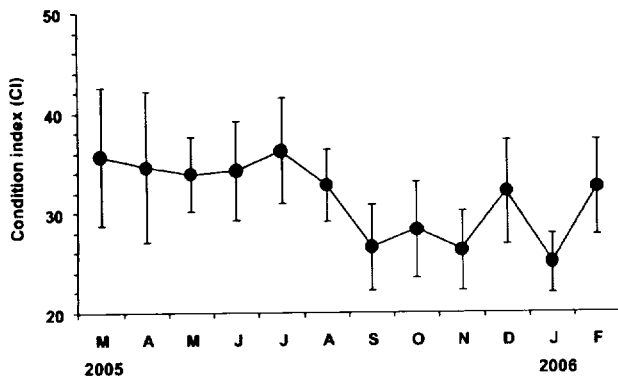


Figure 7. Monthly mean condition index of *F. mutica* recorded from March 2005 to February 2006.

other in September to October. Yoshida (1953, cited in Chang and Lee (1982)) also reported an *F. mutica* spawning season from June to October in Jinhai Bay on the southern coast of Korea that paralleled the one in Gamakman Bay.

The gametogenesis of *F. mutica* observed in this study in Cheonsu Bay is broadly comparable with the annual reproductive cycle of cockles reported from Gamakman Bay by Chang and Lee (1982). In Cheonsu Bay, the onset of gametogenesis was observed in December, with 2 spawning peaks in an annual reproductive cycle: the first in early summer (June to July) and the second in late summer to early fall (September to October, Fig. 4). The similarities between the annual reproductive patterns of cockles observed in the current study and those reported for Gamakman Bay could be attributed to the similar seasonal surface water temperature ranges, despite the distance between the 2 bays. According to Chang and Lee (1982), the surface water temperature in Gamakman Bay ranged from 4.1°C in January to 23.9°C in August, which is comparable with the annual surface water temperature range recorded in the current study: from 4.8 (February)–25.1°C (August). Along with the surface water temperature, food availability in the water column is also considered to be a major environmental factor governing the annual reproductive cycles of marine bivalves. In Cheonsu Bay, Kim et al. (2005) measured seasonal variations in total chlorophyll level. They reported that the total chlorophyll levels in the bay varied from 1.0 (August)–7.0 µg/L (April). This is somewhat comparable with the chlorophyll levels reported from Gamakman Bay by Hyun et al. (2001), which ranged from 0.5 (August) to 4.0 µg/L (April). Therefore, it is postulated that the similar patterns in annual reproduction observed in Gamakman Bay and Cheonsu Bay are attributable to the similar hydrographic conditions of the 2 bays.

Annual reproductive cycles in marine bivalve reproduction are often inferred from temporal changes in CI (Peterson et al. 2004, Duprat-Bertazzi & Carcia-Dominguez 2005, Herrmann et al. 2009, Kim et al. 2010). Because marine bivalves release vast amounts of germ cells through spawning, the CI declines dramatically after a spawning event (Kang et al. 2003, Park & Choi 2004, Uddin et al. 2007). As shown in Figure 7, CI in this study was found to have dropped considerably from July (36) to September (26), indicating that a mass spawning of *F. mutica* occurred in the bay during this period. CI also increased from November to December, then dropped again (Fig. 7). However,

the increases and decreases in CI observed in November and December were not linked to spawning activity, because most cockles examined in November were in the resting stage (Fig. 4). It is likely that such increases and decreases in CI during reproductively inactive periods are associated with the utilization of stored energy. As Figure 6 demonstrates, total carbohydrate, lipid, and protein levels in the tissue increased from November to December and then dropped from December to January, indicating that the cockles used stored energy during a relatively food-poor period in December and January (Kim et al. 2005).

A few studies also have reported on the annual reproductive cycle of *F. mutica* in eastern Pacific waters. In Tokyo Bay, Japan, Tian and Shimizu (1997) measured monthly changes in the shell length and meat weight of *F. mutica* to determine the spawning period of the cockle in the bay. In their study, the annual highest fatness index (i.e., the ratio of meat weight to SL) was observed in March, and the index declined rapidly from April to June. Based upon this observation, Tian and Shimizu (1997) postulated a single spawning peak for *F. mutica* in Tokyo Bay occurring in May to June. Inoue (1955, cited in Chang and Lee (1982)) also reported 2 spawning peaks for the *F. mutica* population in Seto Inland Sea in Yamaguchi Prefecture, Japan: one in April to May and the other in August to October. Nishihira (1980, cited in Chang and Lee (1982)) also reported 2 spawning peaks for *F. mutica* in the Kyoto area of mid Japan: one in June to August and the other in September to October. The spawning period and the frequency of spawning peaks reported from Japan varied with location, because *F. mutica* in southern Japan may spawn earlier than northern populations. The annual reproductive cycle of *F. mutica* observed in the Kyoto area (Nishihira 1980) is somewhat comparable with the cycle observed in the current study.

Liu et al. (2008b) investigated the annual reproductive cycle and subsequent changes in tissue biochemistry of an *F. mutica* population on the coastal Yellow Sea of China (36°41'–37°36' N, 121°11'–121°42' E). On the coast of Weihai, where the seawater temperature ranged from 1.8 (January)–29.5°C (August) annually, *F. mutica* commenced gametogenesis in November, when the water temperature reached 7.1°C, and a single spawning peak was observed in May to June when the water temperature ranged from 19.4–23.6°C. It is remarkable that the cockle population in Weihai remained sexually inactive (i.e., in the undifferentiated stage) during summer and early fall (August to October (Liu et al. 2008b)). In contrast, studies of the annual reproductive cycles of *F. mutica* carried out in Korea and Japan have reported that cockles were actively spawning or in spent stages during summer or early fall (Nishihira 1980, Chang & Lee 1982).

The observed differences between the reproductive pattern of cockles found in Weihai and in the current study area could be explained in part by the size or age of cockles used in the studies and the level of available food in the environment. According to Liu et al. (2008b), the SL of the cockles collected from the coast of Weihai varied from 47.0–60.5 mm, whereas SL of cockles analyzed in the current study ranged from 62.7–81.0 mm (Table 1). Tian and Shimizu (1997) investigated the relationship between age and SL of *F. mutica* in Tokyo Bay and found that *F. mutica* with an SL smaller than 60.0 mm were considered to be 1 y old or younger, whereas cockles with an SL ranging from 60.0–82.7 mm were considered to be 2 y old. They also suggested that the longevity of *F. mutica* is about 2 y.

Therefore, it is likely that the cockles analyzed in the current study were 2 y old, whereas the cockles collected from the coast of Weihai, China, by Liu et al. (2008b) were 1 y old. It is remarkable that the chlorophyll *a* level reported from the Weihai area in spring and summer was extremely high. Liu et al. (2008b) reported chlorophyll *a* levels in Weihai of 17.7 µg/L in April and 25.8 µg/L in September, which is 2.5–3.5 times higher than the maximum chlorophyll level of 7.0 µg/L reported for Cheonsu Bay by Kim et al. (2005). Therefore, it is postulated that the earlier sexual maturation and subsequent earlier spawning observed in *F. mutica* in Weihai, China, is in part attributable to the high level of available food in the water column and the age of the cockles studied. High levels of food supply may accelerate the rate of gonad maturation, resulting in relatively early spawning. The relatively small size or age of cockles is also believed to be responsible for the earlier spawning of *F. mutica* observed in Weihai, China. According to Hofmann et al. (1992), smaller or younger animals may spawn earlier than larger or older animals, because less time is required for smaller or younger animals to accumulate the gonadal materials necessary to activate a spawning event, as rates of food acquisition decrease relative to biomass at larger sizes more than the costs of respiration. However, further studies of size- or age-dependent spawning or reproductive effort in *F. mutica* should be conducted to substantiate this hypothesis. Alternatively, genetic differences between the 2 cockle populations may result in different annual reproductive patterns, as was observed in the Eastern oyster, *Crassostrea virginica* (Barber et al. 1991).

The annual reproductive cycle of marine bivalves is often closely associated with annual energy storage-consumption rotation (Sastri 1979, Urrutia et al. 1999). Numerous studies have reported that marine bivalves reserve high levels of carbohydrate in their tissues prior to vitellogenesis and that the reserved energy is used or converted into gonad materials such as yolk protein during the active spawning period (Robert et al. 1993, Berthelin et al. 2000, Ojea et al. 2004, Dridi et al. 2007). As shown in Figure 6, the current study found that total carbohydrate levels were significantly higher during spring (March to June, ANOVA, $P < 0.05$), when cockle gonads were in the developing stage. It is remarkable that total carbohydrate levels dropped dramatically from June to August, as the cockles became ripe and spawned during these months. These data suggest that similar to the case in other marine bivalves, carbohydrates are the main reserve materials in *F. mutica*. It is likely that carbohydrates were stored in the tissue during a spring phytoplankton bloom (Kim et al. 2005) and were converted into gonad materials during

vitellogenesis. Liu et al. (2008b) also reported seasonal changes in *F. mutica* tissue carbohydrate levels. In Weihai, China, carbohydrate levels increased rapidly from June to August, when cockles completed spawning and began resting. From November to January, carbohydrate levels dropped remarkably, and this decline coincided with gonad maturation during this period. Liu et al. (2008b) also concluded that carbohydrate (i.e., glycogen) is the main source of energy for gametogenesis in *F. mutica*.

In contrast to carbohydrates, protein levels increased during gonad maturation and the subsequent spawning period from June to September (Fig. 6), and then declined as spawning activity was completed in the fall. Several studies have reported that protein is the major component of marine bivalve oocytes, accounting for 40–50% of egg weights (Lee & Heffernan 1991, Choi et al. 1993, Kang et al. 2003, Park et al. 2003, Park & Choi 2004). Liu et al. (2008b) also analyzed the levels of protein in different tissues of *F. mutica* during the annual reproductive cycle. They observed decreases in protein levels in the foot during the gonad maturation period and postulated that protein is used as a nutrient when carbohydrate reserves become depleted.

Significantly higher levels of ash (ANOVA, $P < 0.01$) were observed during the nutrient-poor period from November to January (Fig. 6). During this period, carbohydrate and lipid levels were also low, indicating that the nutritional status of the cockles was poor during this period.

In summary, the annual reproductive cycle of the cockle *F. mutica* in Cheonsu Bay off the west coast of Korea was investigated using histology. Cockles in Cheonsu Bay spawned from May to September, with 2 spawning peaks during this period: one in June to July and the other in September to October. To enhance cockle resources in Cheonsu Bay, spawning cockles are currently protected from fishery pressure by way of a fishery closure season from July to September. However, histology clearly reveals that a certain proportion of cockles are actively engaged in spawning in June. Therefore, it is recommended that the current fishery closure period be extended to run from June to September to protect spawning cockles in June.

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