Korean J. Malacol. 35(2): 81-86 2019
https://doi.org/10.9710/kjm.2019.35.2.81

[단보, Short communication]

Reproductive condition and fecundity of female Suminoe oyster *Crassostrea ariakensis* (Fujita, 1913) from Seomjin River estuary on the south coast of Korea during post-spawning period

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ABSTRACT

While Suminoe oyster *Crassostrea ariakensis* (Fujita, 1913) occurs widely in subtidal estuaries on the south coast of Korea, few studies have reported their reproduction. In the present study, we estimated reproductive effort of female Suminoe oysters from Seomjin River estuary on the south coast of Korea during spawning and post-spawning. Gametogenic condition was assessed using histology and reproductive effort and/or fecundity was determined using an indirect enzyme-linked immunosorbent assay (ELISA). Histology revealed that at Seomjin River estuary in 2008, the females spawned mostly in August and September. Gonad somatic index (GSI, the reproductive effot) of the female in ripe stage was estimated as 0.346, which was equivalent to 108 million eggs. The potential fecundity of Suminoe oyster determined in this study was smaller than the fecundity reported previously from the same sampling site (162 to 910 million/oyster), possibly due to size of the oysters used in the analysis.

Key Words: *Crassostrea ariakensis*, Suminoe oyster, Seomjin River estuary, Potential fecundity

INTRODUCTION

Suminoe oyster *Crassostrea ariakensis* (Fujita, 1913) occurs commonly in river mouth estuaries on the south coast of Korea, where salinities vary with a great range (Yoo *et al*., 2004; Yoon *et al*., 2008). Suminoe oysters often reach over 20 cm in shell length (SL) within a few years (Harding and Mann, 2006). Due to their size and unique taste, this estuarine oyster was attempted to produce in commercial scale on the south coast of Korea (Yoo *et al*., 2004; An *et al*., 2006). To run a hatchery for the seed production and/or management of the wild population, understanding the annual gametogenesis and subsequent spawning and amount of gamete released to the environment in marine bivalves is crucial (Deslous-Paoli and Heral, 1988; Thompson *et al*., 1996; Gosling, 2003). Currently few studies have investigated reproduction of Suminoe oysters in Korean waters (Yoo *et al*., 2004; Joo, 2006; Yoon *et al*., 2008; Kim and Choi, 2012).

Quantification of reproductive effort in marine bivalves is often hampered due to absence of a distinct gonad, except scallops (see Choi *et al*., 1993 and Kang *et al*., 2003). Choi *et al* (1993) first developed an antibody to the eastern oyster egg (*C. virginia*) protein, which was, in turn, used in quantification of the egg protein in known quantity of oyster tissue homogenate using indirect enzyme-linked immunosorbent assay (ELISA). The ELISA technique was rapid and sensitive

Received: May 17, 2019; Revised: June 21, 2019; Accepted: June 26, 2019
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1225-3480/24727

enough to determine a minute quantity of the oyster egg proteins, regardless of the reproductive stage. Similarly, Kang *et al.* (2003) developed a polyclonal antibody from egg protein of the Pacific oyster *C. gigas* in order to determine the reproductive effort. Kim and Choi (2012) also produced a polyclonal antibody against to the Suminoe oyster egg protein, and they estimated the potential fecundity of Suminoe oysters from Seomjin River estuary on the south coast of Korea.

In 2008, egg mass of Suminoe oysters in ripe and partially spawned stages from Seomjin River estuary was assessed using the polyclonal antibody developed by Kim and Choi (2012). Reproductive conditions of the females collected from the estuary during August and December in 2008 were also analyzed using histology. In this study, we report the potential fecundity and reproductive condition of female Suminoe oysters at Seomjin River estuary.

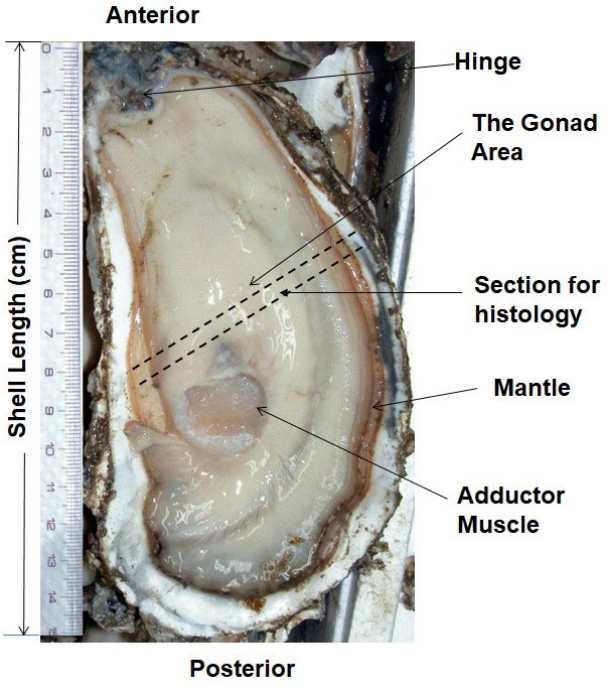

Fig.1. *Crassostrea ariakensis* collected from Seomjin River estuary in 2008.

MATERIALS AND METHODS

1. Sampling effort

For the analysis, adult Suminoe oysters ranging 125 to 155 cm in SL were collected monthly from Seomjin River estuary from August to December in 2008 (Table 1). To evaluate the reproductive condition, 3mm-thick longitudinal section was cut in the middle of the body and fixed in the Davidson's fixative for histology (Fig. 1). The residual tissue was freeze-dried at −75°C and homogenized using a pestle and mortar. Based upon histological appearance of the gonad, reproductive stage (i.e., reproductive condition) of the individual female was categorized as 1) early developing, 2) late developing, 3) ripe, 4) partially spawned, 5) spent/resorbing, and 6) resting (Mondol *et al.*, 2016).

2. Quantification of the egg mass and fecundity estimations

Quantity of the egg protein in an individual oyster as assessed using an indirect ELISA (Kang *et al.*, 2003). In ELISA, the rabbit anti-*C. ariakensis* egg protein IgG developed by Kim and Choi (2012) was served as the primary antibody and goat anti-rabbit IgG alkaline phosphatase conjugate was used as the secondary antibody with ρ-nitrophenylphosphate (ρ-NPP, Fluca) substrate as the coloring agent. Optical density of the final immune-reaction end product in each well was measured at 405 nm using a micro-plate

Table 1. Number and the mean size (shell length in mm) of Suminoe oyster analyzed in this study

Sampling Period (2008)	N	Shell Length (mm)
August	40	125.455
September	40	141.855
October	40	148.51
November	40	160.26
December	40	155.138

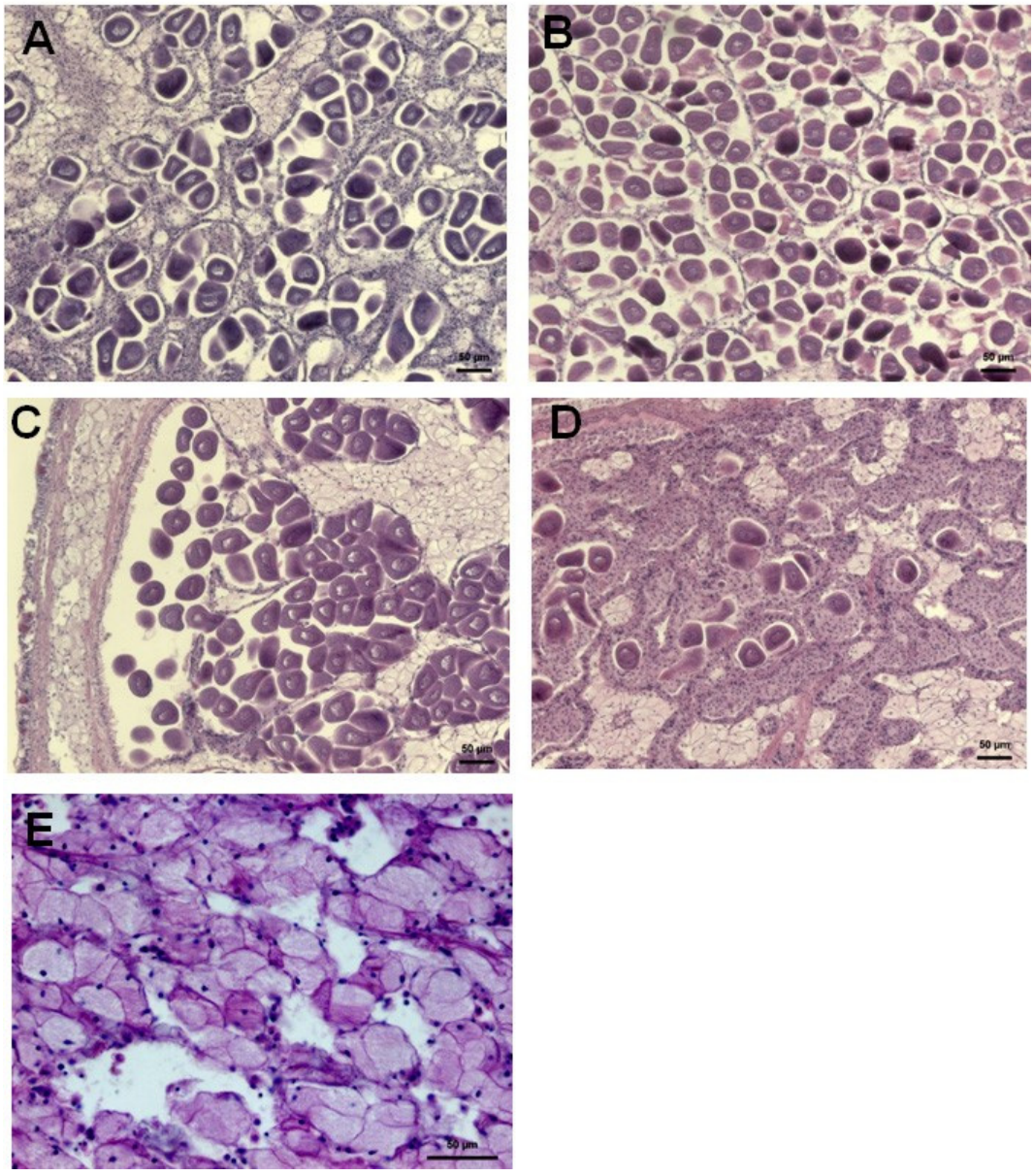

Fig. 2 Micrographs of different reproductive stages of *C. ariakensis* observed in this study. Scale bars = 50 μm. **A**, late developing, **B**, ripe, **C**, Partially spawned, **D**, spent/resorbing, **E**, resting.

reader (Bio-Tek). The quantity of Suminoe oyster egg protein was referred from the standard regression curve (i.e. optical density vs oyster egg protein concentration) constructed from known quantity of the egg homogenate included as positive controls in each ELISA. Finally, we multiplied the constant 1.96 (i.e. egg protein to the total egg mass ratio, see Kim and Choi, 2012) to the egg protein estimated from the ELISA to determine the mass. The potential fecundity was also estimated from the fully ripe, partially spawned and spent/resorbing females. The potential fecundity was estimated by dividing the known quantity of eggs of individual oyster by the weight of single egg, 14 ng (Kim and Choi 2012).

RESULTS and DISCUSSION

1. Reproductive condition of *C. ariakensis*

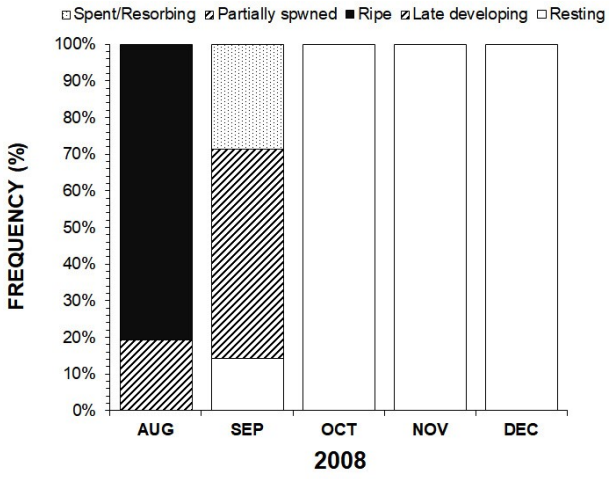

Fig. 3. Frequency distribution of reproductive stage of female *C. ariakensis* analyzed from August to December 2008 at Seomjin River estuary.

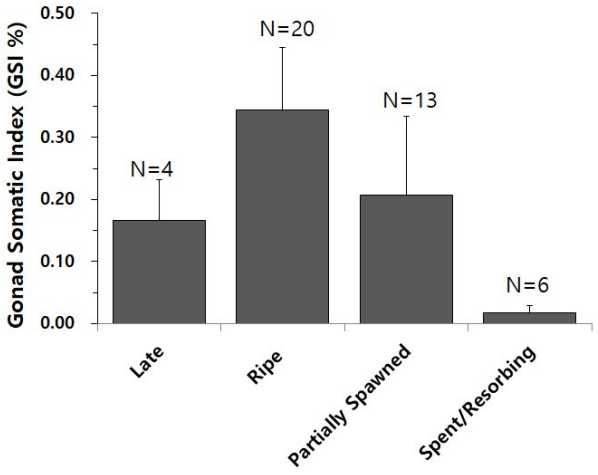

Fig. 4. Gonad somatic index (GSI) of female *C. ariakensis* estimated from different reproductive stages of late developing (late), ripe, partially spawned, and spent/resorbing.

Figure 2 shows frequency distribution of different reproductive stages of the female oysters examined by histology. In August, 80% of the females exhibited fully ripe eggs in the follicles, while 20% of the oysters were engaged in spawning. Proportion of the females in spawning increased from August to September, as 57.1% of the females were in spawning (i.e., partially spawned) in September. In October, all the oysters analyzed and examined were in resting stage, demonstrating empty follicles. Figure 3 shows micrographs of different reproductive condition of the females collected during August and December 2008.

2. Gonad Somatic Index (GSI) and Potential Fecundity

ELISA assessed the egg mass only from the females collected during August and September, as they contained a certain amount of the egg protein in the ripe, partially spawned, and spent/resorbing stages. In contrast, ELISA did not detect the egg protein in the oysters in resting stage during October and December. Gonad Somatic Index (GSI), a ratio of the egg mass to the total tissue weight (dry weight) was calculated to normalize the reproductive effort of the individual female (Fig. 4). In late developing stage, the mean GSI reached 0.167, 0.345 in ripe, 0.207 in partially spawned, and 0.017 in spent/resorbing stage, suggesting that as much as 34.5 % of the female body tissue in ripe stage is composed of the eggs. Mean GSI

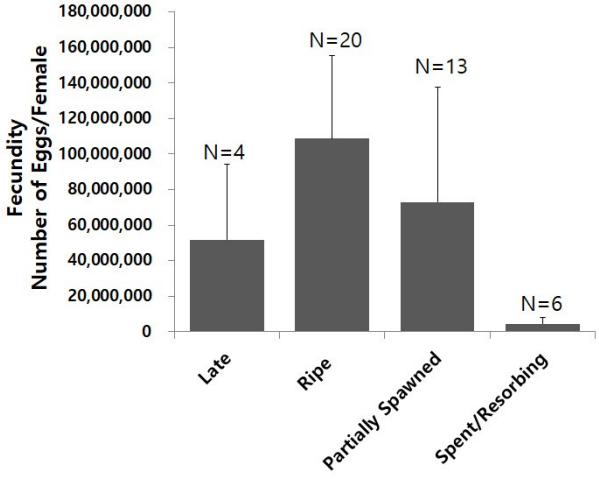

Fig. 5. Potential fecundity of *C. ariakensis* determined from different reproductive stages.

of the oysters in spent stage was determined as 0.017, suggesting the mass of the residual eggs absorbed after completion of reproduction accounts for approximately 1% of the body weight.

Potential fecundity of Suminoe oysters analyzed in this study is presented in Fig. 5. The fecundity in late developing stage was estimated as 51.1 million eggs, while the fecundity increased to 108.6 million eggs in ripe stage then dropped to 64.8 million eggs in partially spawned oysters (Fig. 5).

Table 2 summaries fecundity of oysters reported

Table 2. Fecundity of oysters reported previously from different marine environment

Species	Oyster size (TDWT g)	Method of estimation	Fecundity (million eggs)	Location	Author
C. gigas	1.05 to 3.10	Weight loss before and after spawning	34 to 148	Marennes-Oleron Bay, France	Deslous-Paoli and Heral 1988
C. gigas	0.449 to 5.407	ELISA	4 to 196	Goseong Bay, South Coast of Korea	Kang *et al.* 2003
C. gigas	0.9 to 1.8	ELISA	1.6 to 106.5	Goseong Bay, South Coast of Korea	Ngo *et al.* 2006
C. gigas	4.0 to 8.5	ELISA	90 to 240	Normandy, France	Royer *et al.* 2008
C. ariakensis	10.5 to 13.6	ELISA	162 to 910	Seomjin River estuary, South Coast of Korea	Kim and Choi 2012
C. ariakensis	3.31 to 7.59	ELISA	31 to 225	Seomjin River estuary, South Coast of Korea	Present study

from various studies. As the table shows, fecundity of the Pacific oysters vary from a few million (Kang *et al*., 2003) to 240 million (Royer *et al*., 2008). According to Ngo *et al*. (2006), the Pacific oysters in long-line culture on the south coast of Korea may release as much as 106 million eggs during spawning. Kim and Choi (2012) also estimated fecundity of Suminoe oysters in spawning at Seomjin River estuary using ELISA. Kim and Choi (2012) reported the fecundity of Suminoe oyster as 162 to 910 million eggs, which is comparatively higher than the fecundity estimated in this study (31 to 225 million eggs). The difference in the fecundity between Kim and Choi (2012) and the present study can be explained, in part, by size of the oysters used in the analysis. As Table 2 indicates, size of the female Suminoe oysters analyzed in this study range from 3.31 to 7.59 g in dry weight, which is one half or one-third of the oysters analyzed by Kim and Choi (2012). Several studies have reported that oyster reproductive effort is often size-dependent, as the oyster size increase, the reproductive effort also increases proportionally (Choi *et al*., 1993; Kang *et al*., 2003; Park and Choi, 2004). Kim and Choi (2012) also confirmed the size-dependent reproductive effort of Suminoe oysters at Seomjin River estuary in 2007.

ACKNOWLEDGEMENT

The authors wish to thank the staffs of Shellfish Aquaculture and Research Laboratory of Jeju National University for sampling and histological preparation. This study was supported by 2018 Jeju National University grant.

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