A THESIS FOR THE DEGREE OF
MASTER OF SCIENCE

Temporal variation in the reproduction and tissue biochemical composition in the Gray mussel, *Crenomytilus grayanus* (Dunker 1853) on the east coast of Korea

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August, 2019

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A dissertation submitted in partial fulfillment of the requirement for the degree of
Master of Science

July 2019

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ACKNOWLEDGEMENT

Throughout the writing of this thesis, I have received a great deal of support and assistance. Firstly, I would first like to thank my thesis advisor Prof. Kwang- Sik Choi, School of Marine Biomedical Science at Jeju National University, for the continuous support of my M.Sc. study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

I would like to express my sincere gratitude to experts who were involved in the validation of this research project: Prof. Kyeong-Jun Lee and Prof. Suk-Geun Jung, School of Marine Biomedical Science at Jeju National University. Without their passionate participation and input, the validation could not have been successfully conducted.

I would like to thank all my lab members Dr. Hyun- Sil Kang, Dr. Hyun- Ki Hong, Miss. Hye- Mi Lee, Mr. Young- Ghan Cho, Miss. Harshani Deepakumari, Mr. Jong Seop shin and Miss. Jeong Hwa Kim, whom I have had the pleasure to work during this and other related projects, specially Dr. Hee-Jung Lee and Dr. Hee-Do Jeung for their encouragement and help me to be successful of my research. It was great sharing laboratory with all of you during last two years. I would also like to acknowledge Mr. Ron. G. Noseworthy for the proofreading of this thesis, and I am gratefully indebted to his for his very valuable comments on this thesis.

A very special gratitude goes out to all down at BK 21 PLUS research fund and Ministry of Education, Korea for providing the funding for the work.

I express my sincere gratitude to Prof. Mahanama De Zoysa and Dr. Janak K. Vidanarachchi for introduce me to Prof. Kwang- Sik Choi. A very special thanks goes to Dr. (Miss) J. Sinniah, Former Head of the Department, Animal Science and Prof. M. Thushiyanthi, Former Dean Faculty of Agriculture, University of Jaffna, to support and guidance to get my study leave during this study.

Finally, I must express my very profound gratitude to my parents and to my girlfriend for providing me with unfailing support and continuous encouragement throughout my years of study.

ABSTRACT

The Gray Mussel *Crenomytilus grayanus* occurs in high density in the shallow, rocky, subtidal zone on the east coast of Korea, where this mussel is commercially exploited by the local shellfish industries. Despite its commercial and ecological importance, little is known about its reproductive physiology, which is crucial to the management and the future aquaculture development of this species. In this study, we first examined the annual reproductive cycle and temporal changes in the tissue composition of *C. grayanus* during September 2012 to August 2013 from the east coast of Korea. Histology revealed that oogenesis and spermatogenesis commenced in September, as the small oogonia (16.67 ± 4.87μm) and spermatogonia could be observed in the follicles, respectively. Mature oocytes (46.55 ± 9.91 μm) and mature spermatozoa were observed in March, and most of the males and females spawned during May and June, when the surface seawater temperature (SST) increased from 15.5 to 22.3ºC. The total carbohydrate level in the tissue increased dramatically from April to May, which coincided with the chlorophyll a maximum occurring in April. Condition index, as a ratio of tissue weight to shell weight, also increased from February to May, and then declined in June, indicating that most mussels spawned during May and June. Our data suggests that *C. grayanus* is a spring spawner, and the onset of gametogenesis and subsequent spawning is closely linked to the seasonal changes in water temperature and food availability in the water column.

Keywords: annual gametogenesis, *Crenomytilus grayanus*, East Coast of Korea, mussel, spring spawner

1. INTRODUCTION

Mussels are important shellfish resources which exceed their commercial value as a source of protein and a potential candidate for aquaculture in many countries (Gosling 2003). The global mussel production has increased gradually over the past decade, from 1.77 million metric tones (MT) in 2006 to 2.1 million MT in 2016 (FAO FISHSTAT). In Korean waters, several species of native and introduced mussels have been exploited commercially, while the introduced Mediterranean mussel *Mytilus galloprovincialis* has been farmed exclusively, mostly in small bays on the south coast of Korea. The annual landings of mussels in Korea have ranged from 40 thousand to 100 thousand MT over the past decade (FAO 2016), and the landings originated mostly from the mussel farms located on the south coast. *Crenomytilus grayanus* is a macro benthos, a large bivalve of the family Mytilidae, commonly found subtidal area in East Sea (Scarlato1981, Galysheva 2008, Selin 1991, Selin and Dulenina 2012). Recent studies reaffirmed that *C. grayanus* belongs to family Mytilitade, subfamily Mytilinae (Kartavtsev Yu. Ph., 2018). The *C. grayanus* is also commonly widespread in the East Sea, from coastal area of Gyeongbuk, Korea to Tatarsky Strait, Russia (Lutaenko and Noseworthy 2012, Selin 2012). On the east coast, the mussel, *Crenomytilus grayanus* occurs predominantly on shallow, subtidal, rocky substrate (Scarlato, 1981; Selin 1991; Galysheva 2008; Lutaenko and Noseworthy 2012; Selin and Dulenina, 2012). Due to the high nutritional values and marketability, *C. grayanus* is considered to be an aquaculture candidate species; it is exploited on a small scale by the local villagers on the east coast.

East sea ecosystem is a dynamic environment, largely influenced by Tsushima current pass through Korea Strait which delivers heat and other substances to East Sea

and seasonal changes often synchronized with available food (Onitsuka 2007). The annual average surface seawater temperature (SST) ranged from 10^{o}C to 25^{o}C and salinity varied from 28 to 38 PSU in the east sea of Korea which are suitable and increase the growth rate of *C. grayanus*. (Selin and Dulenina 2012). Currently, the Yesso scallop *Patinopecten yessoensis* is only the shellfish species cultivated on the east coast, using a suspended long-line (Uddin et al. 2007). Aquaculture programs create demands for new mussel species and sites for development and would be of interest to the local population; however, poor management practices, such as overexploitation, would result in unsustainable production (Lee et al. 2011; Yang et al. 2011; Mok et al. 2014).

Bivalve molluscan in the east sea well studied, engaged in crucial role in the development and exploitation of marine resource in Korea. There are several bivalve species such as *Mytilus galloprovincialis*, *Mytilus coruscus*, *Musculus glacialis*, *Musculus koreanus*, *Musculus cupreus*, *Vilisina pillula*, *Adula schmidtii*, *Setifer virgatus* and others which belong to family Mytilidae can be found abundantly in coastal area of Gangwon, Korea (Kulikova 2011; Lutanenko and Noseworthy 2012). The gametogenesis of mussels belongs to Mytilidae family such as *Mytilus coruscus* and other bivalves have been studied well in east sea of Korea. (Kulikova 2011). Understanding the reproductive behavior of marine mussels is important, not only for studying the dynamics of the species but is also one of the key factors in developing a successful aquaculture industry. Mussel may show seasonal or continuous reproductive cycle, mussel which had seasonal reproductive cycle closely linked to water temperature and photoperiod (Ceballos et al. 2000). Valencia et al. (2004) and Fontan et al. (2008) indicated that temperate region located at mid- latitude showed a

remarkable seasonality in annual seawater temperature and it strongly related to atmospheric temperature. The annual reproduction associated with spawning and energy storage in marine bivalves (Urrutia et al. 1999; Yang et al. 2011), is commonly influenced by environmental parameters and food availability (Bayne 1976; Kang et al. 2009). The biochemical composition of marine bivalves varies greatly within a species due to food availability, nutrient composition, seasonal climatic changes, geography, aquaculture methods, and stages of maturation (Kang et al. 2009; Kang et al. 2010; Yang et al. 2011; Uddin et al. 2007). Energy storage and mobilization are key factors that group marine bivalves into two categories based on energy storage and consumption pattern. Conservative species use energy already stored in body parts during reproduction, and opportunistic species use energy from recently consumed food for reproduction (Bayne 1976).

Currently, *C. grayanus* is an ideal candidate for aquaculture on the east coast of Korea. Despite its abundance and popularity, no studies have been carried out on the annual reproductive cycle of *C. grayanus* in Korea. A reproductive study of this species provides essential information which can enhance the wild populations through strategic management planning, such as the best time for seed collection and the environmental conditions needed to induce spawning. The present study focuses on the temporal variations in reproduction and changes in biochemical composition during the reproductive cycle of *C. grayanus* in Korea's eastern coastal region.

2. MATERIALS AND METHODS

2.1. Sampling effort and environmental factors

For histology and biochemical tissue analysis, ***C. grayanus*** mussels were collected monthly by SCUBA from a depth of 2-3 m near Gangneung (37º 45' N, 128º 59' E), on the east coast of Korea, from September 2012 to August 2013 (Fig. 1). Thirty adult mussels with shell length (SL) ranging from 88 to 107 mm were collected monthly (Table 1). Sea surface temperature (SST) and chlorophyll a data were obtained from the Giovanni online data system (NASA GES DISC, https://giovanni.gsfc.nasa.gov/giovanni). SST in the study area ranged annually from 9.5 in January to 29.5ºC in August, while the chlorophyll a level varied from 0.35 µg/l in June to 1.8 µg/l in April (Fig 2).

Fig. 1 Location of study area on the east coast of Korea.

Table 1 Summary of sampling effort. The values represent the monthly mean and standard deviation. N- Numbers of mussels used in analysis: SL- standard length in mm.

Month		N	SL (mm)
			mean ± SD
2012	S	30	107.0 ± 8.49
	O	30	99.2 ± 7.88
	N	30	99.8 ± 8.05
	D	30	91.3 ± 5.23
2013	J	30	100.1 ± 5.68
	F	30	95.6 ± 8.64
	M	30	96.2 ± 7.17
	A	30	88.3 ± 6.17
	M	30	95.9 ± 9.12
	J	30	100.8 ± 4.58
	J	30	93.7 ± 6.24
	A	30	89.4 ± 10.85

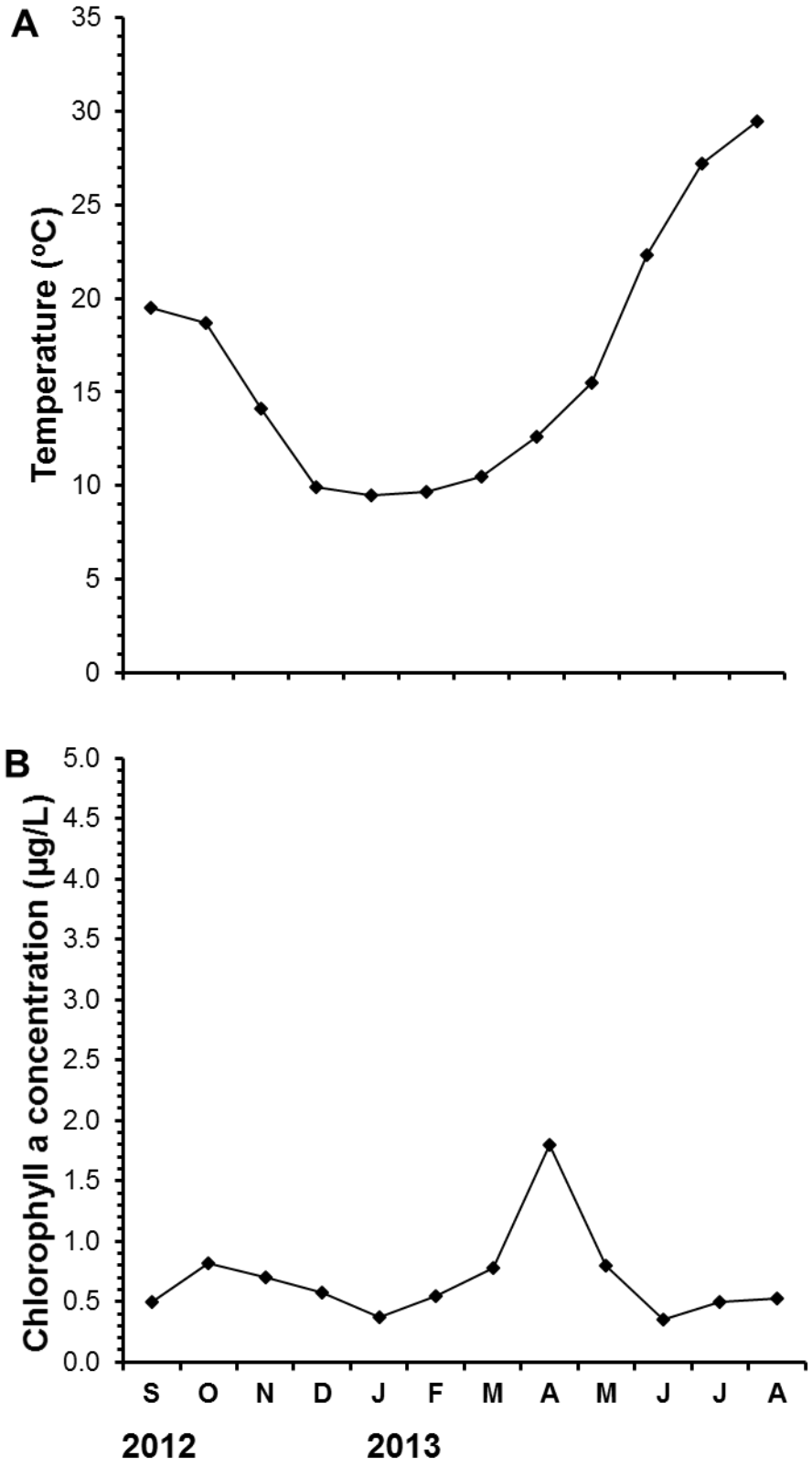

Fig. 2 Seasonal variation in surface seawater temperature (A) and chlorophyll a concentration (B) in Gangneung.

2.2.Reproductive Biometric measurements and histology

After recording the SL, the soft tissue was removed from the shell and weighed by mg using an electronic balance. The dry shell weight was also measured by mg. For histology, a 2 to 3 mm thick section was removed dorso-ventrally from the middle of the body and fixed in Davidson's fixative. The tissue sections were dehydrated using an ascending series of alcohol and embedded in paraffin, the remaining tissues were lyophilized and homogenized for study. The paraffin blocks were sliced to a thickness of 6 μm, stained with Harris's haematoxylin, and counterstained with eosin Y. Microscopic images of the gonad appearing in different reproductive stages were digitized using a digital camera connected to a microscope. Reproductive maturity of gonads was grouped into six stages according to Mondol et al. (2016), as 1) indifferent (i.e., resting), 2) early developing, 3) late developing, 4) mature, 5) partial spawning and 6) spent. The sequences of reproductive maturity of spermatogenesis and oogenesis based on histological changes in the gonad condition are described in Table 2. Oocyte diameter was measured from the digitized microscopic images of the gonads using ImageJ software (National Institute of Health, USA). The remaining tissues were freeze-dried and weighed by mg using an electronic balance. Temporal changes in the tissue measured by condition index (CI), a ratio of tissue dry weight to shell dry weight. The remaining tissue which was used for histology weighed, freeze-dried and stored at -70^{o}C. The shells were dried (50^{o}C) and weighed to calculate the CI.

$$CI = (\text{Dry tissue weight} / \text{Shell dry weight}) \times 100$$

Table 2 Reproductive maturity of spermatogenesis and oogenesis in *Crenomytilus grayanus* based on histological changes in the gonad

Maturity stage	Male	Female
Indifferent	The sex cannot be recognized, and numerous connective tissues can be observed in this degenerative state. Shrunken gonad with no spermatogonia can be observed.	The sex cannot be identified, gonads have shrunken and less activity after spawning. There is no oogonia on follicle wall and more connective tissue can be observed
Early developing	Numerous spermatogonia and few spermatocytes along the follicle wall and contains small size tubules	Numerous primary oogonia and developing oocytes could be seen on thick follicle wall
Late developing	The lumen of the follicle is gradually filled with spermatogonia then spermatocytes and spermatids. Volume of tubules gradually increased, and wall of the follicle become thinner	Oogonia and developing oocytes grow along the wall of the follicle and deposition of egg yolk can be seen, follicles become pear shape
Mature	The diameter of seminiferous tubules further increased and filled with mainly spermatozoa and all other spermatogenic stages. Transformation of spermatids to spermatozoa can be seen clearly due to free active tail.	Mature oocytes which are not packed tightly, found in the lumen of the follicle. Follicles have maximum volume and wall of the follicle become round.
Partially spawning	The central part of lumen becomes empty due to release of spermatozoa. Testicular tubules attained their maximum diameter, follicles become enlarge and few phagocytic cells have found.	Some ova have polygonal shape due to pressure exerted by follicle wall. More free oocytes found in the lumen of the follicle. Specially ruptured follicle wall could be seen.
Spent	Some spermatozoa remain in the follicles and increase connective tissue inter lobular space in the lumen. It is possible to identify the sex of male by unreleased gametes and residual mass. The follicles have shrunken and can be observed phagocytes within the gonads.	Some oocytes which are not fully developed remaining in the lumen therefore sex determination is still possible. The volume of follicles is reduced, and wall has ruptured. Some phagocytes can be observed

2.3.Total carbohydrate and total protein analysis of tissue

The freeze-dried tissue of the mussels, stored at -70^{o}C, was homogenized to determine the total carbohydrate and total protein levels. The total protein in the tissue was determined by the method adopted from Lowery et al. (1951), after the extraction with 0.1 N NaOH at 37^{o}C for 2 hours, bovine serum albumen was used as a standard. Total carbohydrate in the tissue was assessed using the phenol-sulfuric method embraced from Dubois et al. (1956) with dextrose as the standard material. The quantities of protein and carbohydrate were expressed as the percentage of tissue dry weight.

3. RESULTS

3.1.Microscopic observation of gonads

In the sexually indifferent stage, no gametogenic cells could be found along the follicle walls (Fig 3(a), Fig 4(a)) thereby could not differentiate female and male. In early developing stage the follicles are increased in volume and developed oogonia (Fig. 3(b)) and spermatogonia (Fig. 4(b)). The late developing stage female and male follicles were greatly expanded and could be observed larger oocytes, connected with thin stalk with the follicular wall (Fig. 3(c)) while spermatocytes (Fig. 4(c)) clearly identified. The mature stage contained fully compacted by mature oocytes (Fig. 3(d)) while spermatozoa were arranged the tubules toward the centre and spermatocyte and spermatozoa were identified in male (Fig. 4(d)). Loosely packed, free-floating ripe oocytes (Fig. 3(e)) and mature spermatozoa (Fig. 4(e)) could be identified in spawning female and male respectively. Almost all the gametes have been released during the spent stage (Fig 3(f), Fig 4(f)) however follicles often contained residual gametes which were smaller and ruptured.

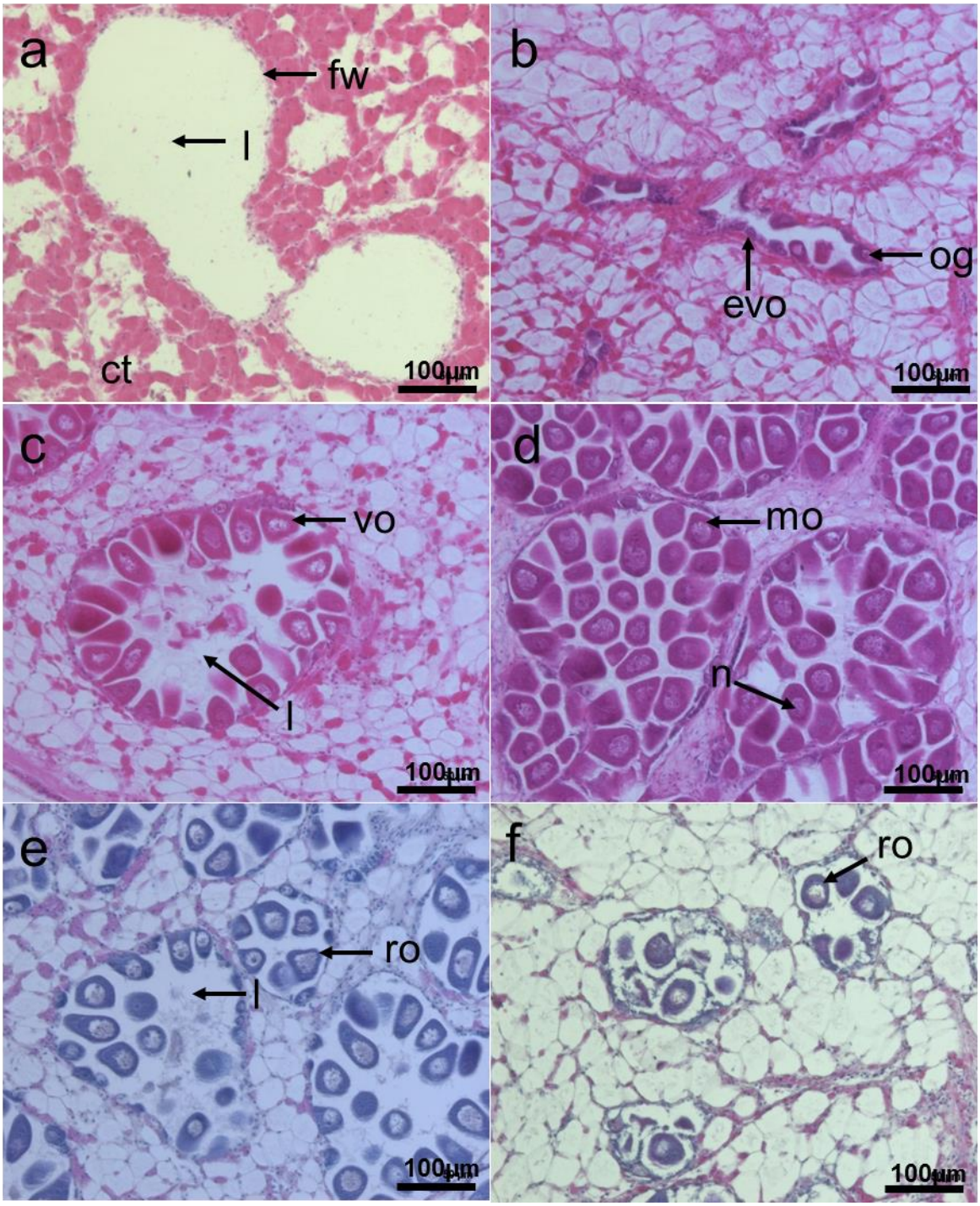

Fig. 3 Photomicrographs of female *C. grayanus* gonads. a- Undifferentiated stage; b- Early developing stage; c- Late developing stage; d- Mature stage; e- Partially spawned stage; f- Spent stage; fw- Follicle wall; evo- Early vitellogenic oocytes; og- Oogonia; vo- Vitellogenic oocytes; mo- Mature oocyte; l- Lumen; ro- Relict oocyte

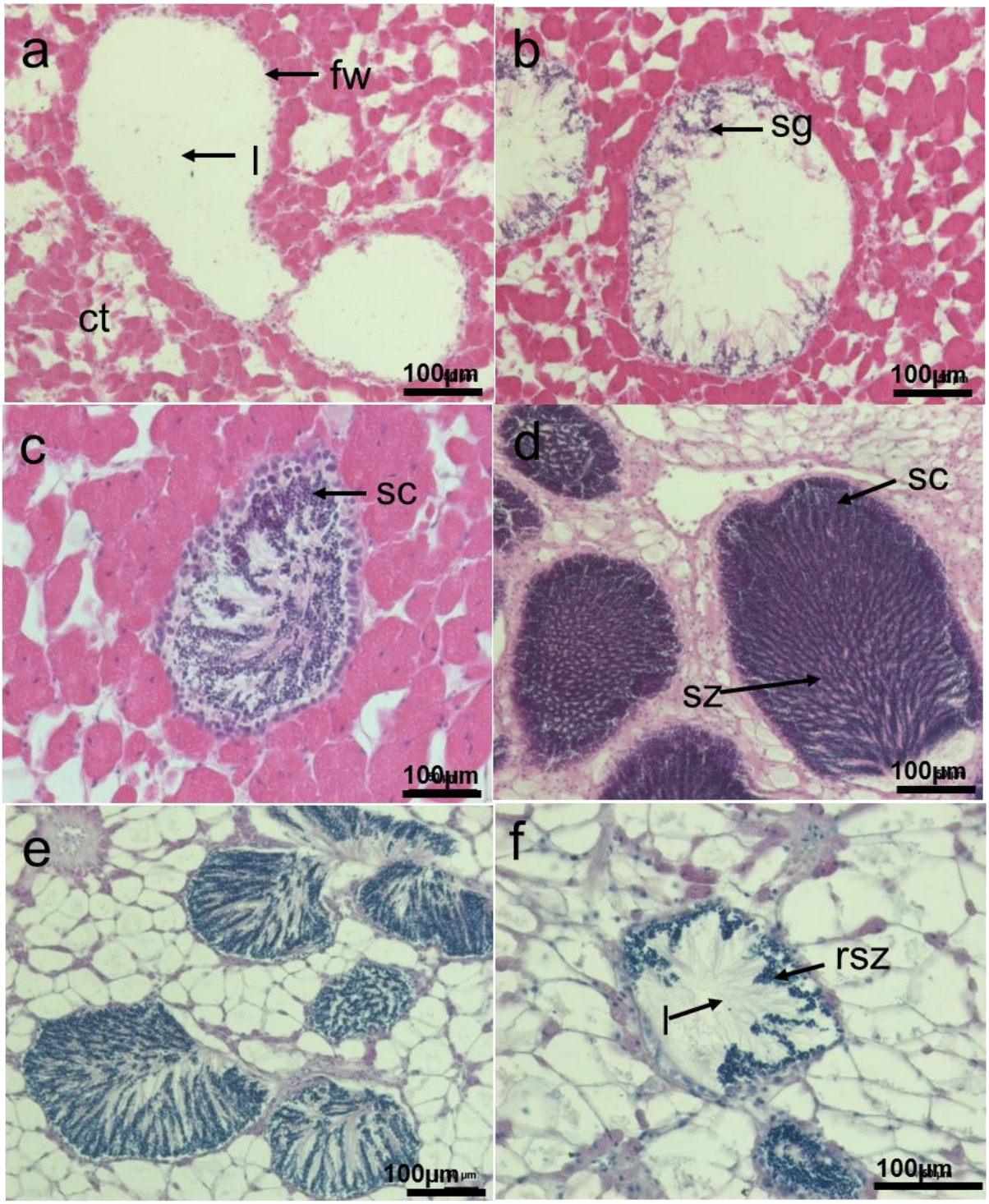

Fig. 4 Photomicrographs of male *C. grayanus* gonads. a- Undifferentiated stage; b- Early developing stage; c- Late developing stage; d- Mature stage; e- Partially spawned stage; f- Spent stage; fw- Follicle wall; sg- Spermatogonia; ct- Connective tissue; sc- Spermatocytes; sz- Spermatozoa; l- Lumen; rsz- Relict spermatozoa

3.2.Annual gametogenesis cycle

Figure 5 shows the monthly changes in gametogenesis observed in females and males. Histology revealed that sexually indifferent mussels could be observed from August to September. Oogenesis and spermatogenesis commenced in September; early developing stages were 34.8% and 28.6% of females and males, respectively, and the SST was 19.5^{o}C. The proportion of females and males in late developing stage increased steadily from October, 2012 to February, 2013, and ranged from 9.1 – 100% and 45 – 85.7%, respectively. Dominant mature oocytes were found in March and April (63.6%); however mature spermatozoa were observed one month earlier, ranging from 14.3 – 58.8%. Partial spawning activity of females and males appears to be synchronous with an annual peak in May, at 54.5% and 41.2%, respectively, when the SST increased to 15.5^{o}C. Interestingly, spent females and males were initially identified in May, at 5.5% and 58.8%, respectively, indicating that some partial spawning had already occurred during this month. Furthermore, partial spawning activity was reduced in June to 9.1% and 10.5% in females and males, respectively, suggesting that most spawning occurred in May. In June, most mussels were in the spent stage, with females at 90.9% and males at 89.5%. The annual reproductive cycle of both female and male *C. grayanus* at Gangneung could be summarized as sexually indifferent from August to September, early developing from September to October, late developing from October to February, mature stage from March to April, spawning in May and June, and spent in June.

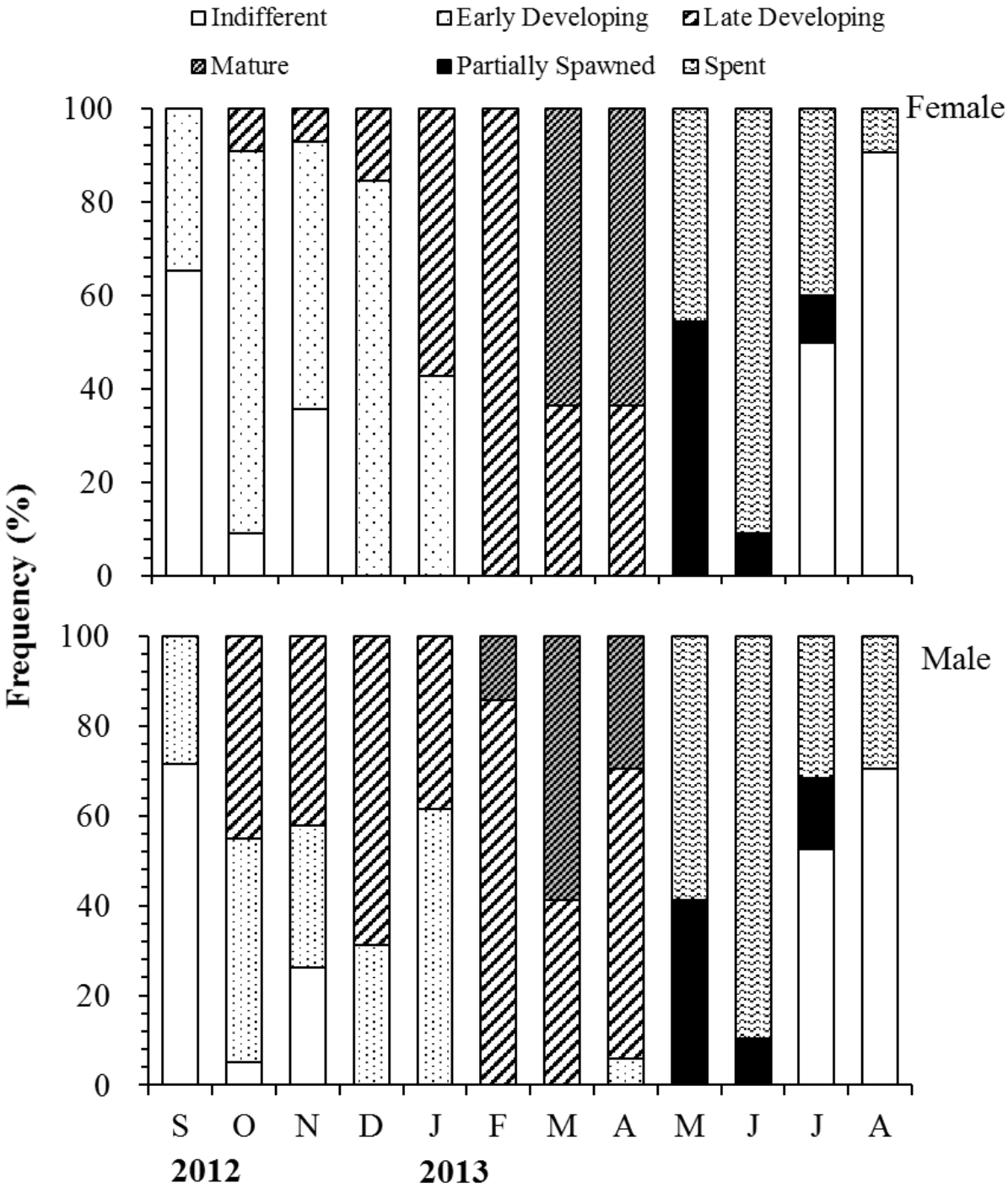

Fig. 5 Frequency distributions of gametogenic stages of female and male mussels.

The monthly variation of oocytes size could be observed during the study period. The class cohorts showed a clear growth progression of oocytes (Fig 6). Oocyte diameter ranged from 10.50μm to 68.84μm, with the monthly mean size ranging from 16.67 ± 4.87μm in September, to 50.39 ± 8.82μm in July, and only a small number of oocytes were larger, > 65μm. Small oocytes, 10 – 30μm in diameter, were dominant in the early developing stage, from September to October. Late developing oocytes measured 25 – 45μm in diameter from late fall to winter. In early spring, from March to April, the dominant oocyte diameter was 50 – 55μm, and most oocytes were in the mature stage. The largest average oocyte size was 50 –55μm, when mussels reached the peak of spawning and, soon after spawning, a smaller number of oocytes were found in the spent stage.

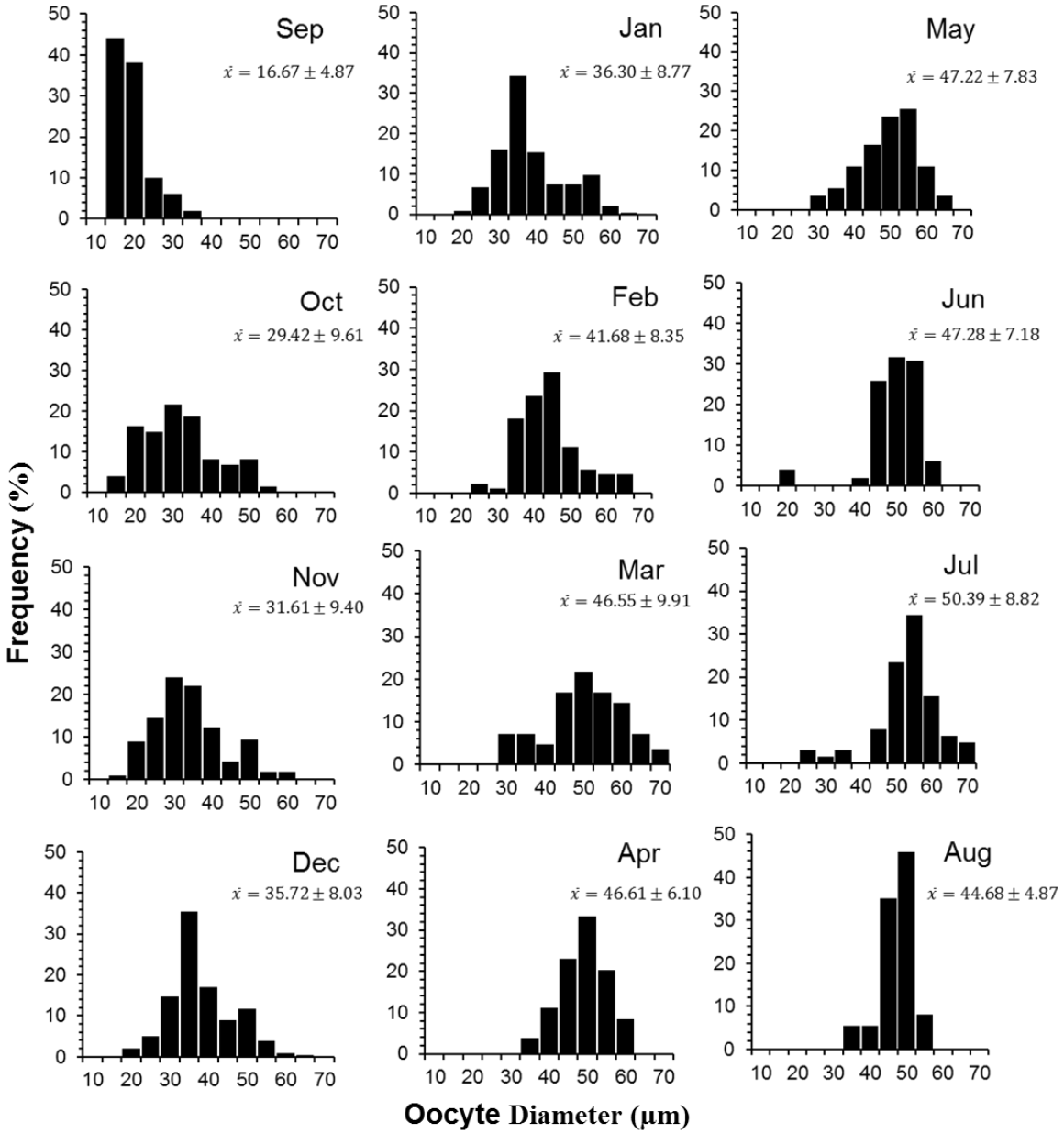

Fig. 6 Frequency distribution of oocyte diameter size classes.

3.3.Total carbohydrate and total protein analysis

Figure 7 plots the seasonal changes of total carbohydrate and total protein content in the dried tissues. The total carbohydrate content of the tissues ranged from 86 – 392 mg/g. A noticeable reduction was observed in total carbohydrates from November (276 mg/g) to January (86 mg/g), coinciding with the decline of chlorophyll a during this period. From winter to early spring, carbohydrate levels were relatively minimal; subsequently, the carbohydrate level increased from April to May, coinciding with an exponential increment of chlorophyll a level during this period. There was a clear seasonal fluctuation in carbohydrate content in the dried tissue of mussels during the study period. In contrast, the protein level of dried tissue was stable, and showed a smaller seasonal fluctuation during this period. The protein content ranged from 290 mg/g in November, to 382 mg/g in September.

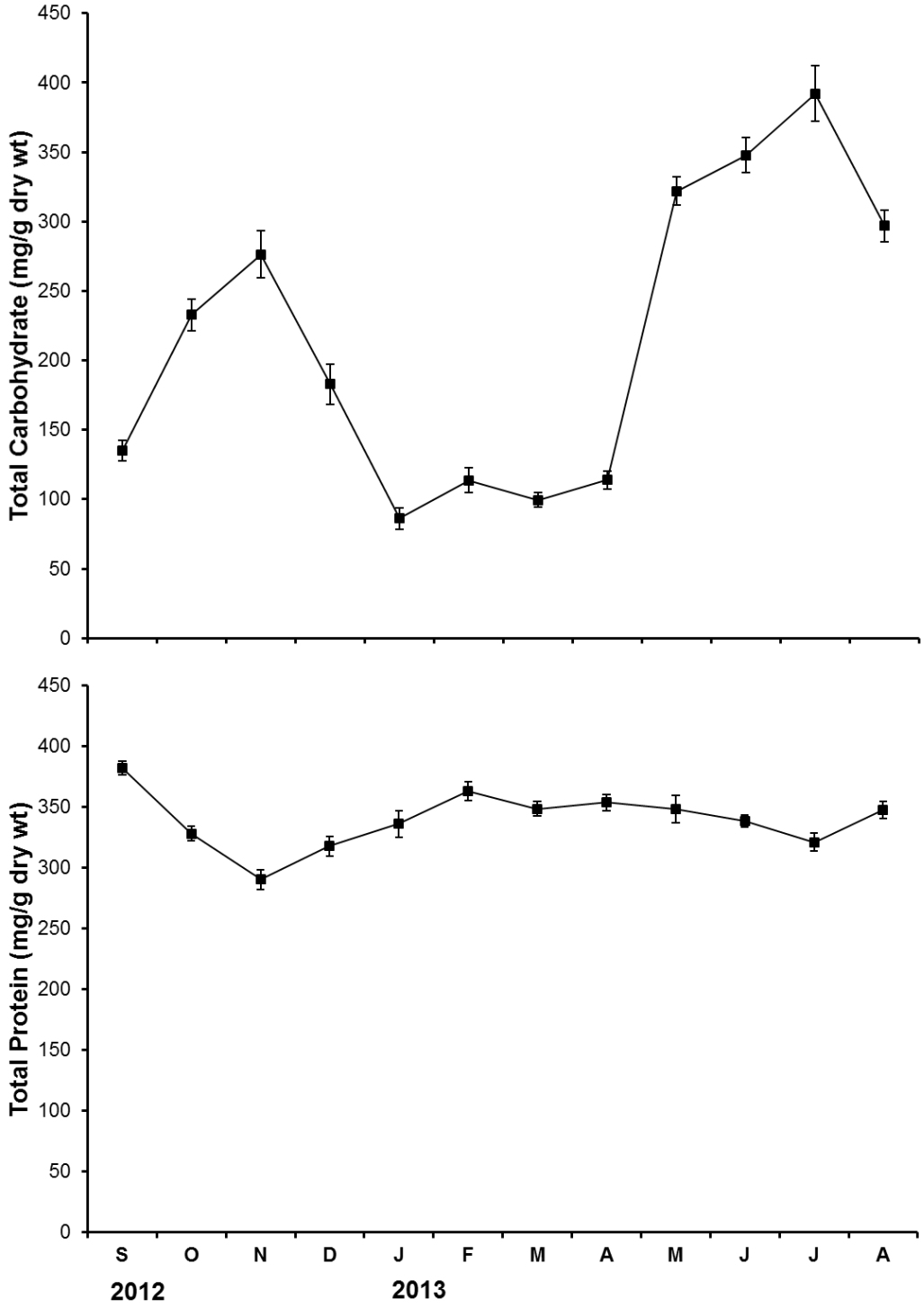

Fig. 7 Seasonal changes in total carbohydrate, and total protein in *C. grayanus* over the study period. The values represent mean ± standard error (SE). The vertical bar in each value represents the standard error.

3.4.Condition Index

Figure 8 illustrates the monthly variation in condition index (CI) of the mussels. The CI ranged from 8.4 – 12.3 in January and July, respectively; it increased from February to March as mussels produced sexually mature gametes. However, on most occasions the CI fluctuated over the period, increasing from January to March, reaching a peak in July, and dropping in September, not being associated with spawning activity and the reproductive cycle. The CI was likely associated with total carbohydrate and total protein content in this study. Higher CI values were observed from March to August, ranging between ~11 and 12, when the carbohydrate level was at its peak.

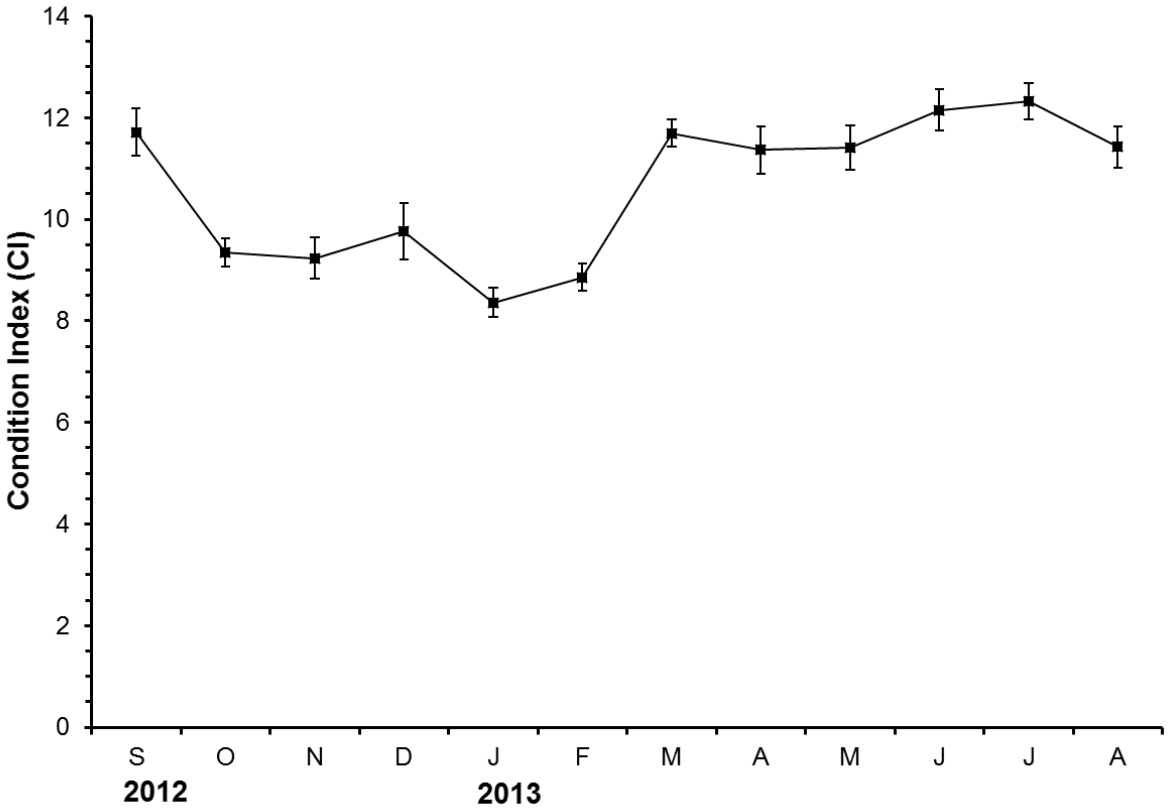

Fig. 8 Seasonal changes in CI in *C. grayanus* over the study period. The values represent mean ± standard error (SE). The vertical bar in each value represents the standard error.

4. DISCUSSION

It is crucial to understand the reproductive pattern and ecology of commercially important mussels to determine the optimum time for seed collection from natural habitats and to initiate successful hatchery or culture practices. Detailed information on reproductive stages can be obtained by histology, which is one of the reliable and successful methods used to monitor the annual reproductive cycle of marine bivalves at regular intervals throughout the year. The reproductive cycle can be grouped into several stages based on the appearance of gametes via histology; however, this classification may be subjective (Gosling 2003; Joaquim et al. 2008; Srakaew et al. 2010). Usually in bivalves, environmental factors influence on gametogenesis, particularly, in the temperate region bivalves are exposed to seasonal variation in temperature and food (Fearman and Moltschaniwskyj 2010). *Crenomytilus grayanus* on the east coast showed gametogenesis equivalent to that of *Mytilus coruscus* as reported by Yang et al. (2015) in the East Sea. In this study, *C. grayanus* on the east coast initiated the gametogenesis in September when water temperature was 19.5^{o} C. Yang et al. (2015) reported that *Mytilus coruscus,* also in the Mytiiidae, commenced its one set of gametogenesis in September, in the East Sea, when the temperature reached 23.7^{o}C. This observation is similar in East Sea, despite of the different species most of the bivalves prospected one set of gametogenesis in Autumn (Uddin et al. 2007; Yang et al. 2015), suggesting that temperature reduction soon after the summer may stimulate gametogenesis. Similar observation was found in *Mytilus edulis* and *Mytilus galloprovincialis,* that gametogenesis was induced in autumn and winter when water temperature dropped after summer in Tasmania (Dix and Ferguson 1984) and Northern Adriatic Sea (Hrs- Brenko 1971).

According to this current study, gametes of *C. grayanus* remained in the late developing stage from October to February (Fig 3), probably due to certain environmental conditions affecting the further development of gametes. Azpeitia et al. (2017) reported that a cycle of gametogenesis of *M. galloprovincialis* along the Basque coast commenced in winter; mature gametes remained until spring, until favorable environmental conditions, such as temperature occurred. Contrary to above observations, Fearman and Moltschaniwskyj (2010) reported that low temperature (10^{o}C) increases the rate of gametogenesis in *M. galloprovincialis*. However, group of bivalves in low temperature with low energy reserve showed slower rate of gamete maturation compared to group with higher energy reserve (Chavez-Villalba et al., 2003), suggests the rate of gamete maturation is not entirely depend on temperature. In current study, minimal total carbohydrate and lower chlorophyll a level have been found throughout winter period, suggests low rate of gametogenesis during developing stage. In this study, spawning mussels were observed in May when the SST was 15.5 ^{o}C, suggesting that *C. grayanus* is a spring spawner. In spite of the different species, the annual reproductive cycle of most of the bivalves in the East Sea exhibited spring spawning activity (Uddin et al. 2007; Yang et al. 2015), suggests environmental factors such as temperature, availability of food and salinity, may stimulate spawning in those bivalves (Bayne 1976). The spring spawning of bivalves in East Sea is believed to be linked to food availability and temperature which are privilege to larvae, as Bayne (1976) stated in most of the bivalves in temperate region. In table 3, some published data of gametogenesis for various mussels are summarized which clearly shows the duration of the gametogenic cycle, gonad development, and time of spawning of bivalves showed wide differences due to environmental condition and food availability in the temperate region (Seed 1976; Villalba 1995; Mugica et al. 2015). *Crenomytilus*

grayanus seems to regenerate their gonads within a shorter period due to temperature reduction as well as with the help of existing energy reserve, accumulated during spring and summer. Similar observation reported in *M. edulis* and *M. galloprovincialis* that consecutive gametogenesis commenced in September within a month of indifferent phase in Northern Adriatic Sea (Hrs-Brenko 1971). The annual gametogenesis of male and female *C. grayanus* exhibited a synchronized pattern of reproductive cycles; a similar observation has been reported by Razek et al. (2014) in both sexes of *Modiolus auriculatus* (Eared Horse Mussel) which showed a similar gametogenesis pattern. At the same time Yang et al. (2015) reported that male *Mytilus coruscus* commenced spawning on the east coast of Korea one month earlier than females, in March. Female mussels were usually slightly behind in the stages of gametogenesis, compared to males at any stage of gametogenesis, due to a higher rate of sperm production compared to ova which needed to produce a large yolk reserve (Seed 1969). Generally, mussels adapt their reproductive cycle based on prevailing environmental conditions, because of the variability of sites and years. The reproductive cycle of mussels may not follow a consistent pattern; therefore, more data is needed to study the gonadal development of mussels (Seed 1976; Azpeitia et al. 2017).

Table 3 Summary of reproduction pattern of Mytilus species reported from temperate region

Species	Oogenesis		Spawning		Oocyte size	Location	Reference
	Month	SST	Month	SST			
M. coruscus	Nov	~17	Feb–Apr	<10	60–80	Gyeokpo, Korea (35º 37'N, 126º 27'E)	Lee et al. (2007)
M. coruscus	Nov	~14	Feb– Mar	~11–12	60–80	Hansan Bay, Korea (34º 38'N, 128º 33'E)	Wi et al. (2003)
M. coruscus	Sep	29.1	Mar	12	67	Nanji Island, China (27º 27'N, 121º 05'E)	Zhu et al. (2018)
M. coruscus	Sep	23.7	Mar– Apr	10.2–12.6	NA	Ulleung Island, Korea (37° 29' N, 130° 48'E)	Yang et al. (2015)
M. edulis	Nov	~9	Apr–Jun	6–14	78	Helgoland, Germany (54° 10'N, 7° 53'E)	Sprung (1983)
M. edulis	Nov	~10	Apr	~9	NA	Long Island, USA (40° 34' N, 73° 33'W)	Newell et al. (1982)
M. edulis	NA	NA	Jul	NA	62	Newfoundland, Canada (48° 47' N, 55° 7'W)	Toro et al. (2002)
C. grayanus	Sep	19.5	May–Jul	15.5	47	Gangneung, Korea (37º 45' N, 128º 59' E)	Present study

Various studies have reported that measuring oocyte size has been widely used in reproductive research (Choi et al. 2002; Kang et al. 2009; Kim et al. 2010; Yang et al. 2017). According to the frequency distribution of oocyte diameters, gametogenesis of *C. grayanus* began in September, when initial smaller oocytes (diameter 10 –15μm) were noticed. Sastry (1979) has reported that initiation of oocyte growth has been influenced by temperature, by regulating transfer of nutrients from reserves to the gonads, thereby increasing oocyte size. The effect of temperature on the rate of gametogenesis is impacted by energy balance. Low temperature accelerates gametogenesis, and at higher temperatures, gametogenesis is slowed by increased energy requirements for metabolism, thereby restricting energy for reproduction in mussels. The rate of gametogenesis in *M. galloprovincialis* decreased as temperature increased, greater than expected frequency of vitellogenic oocytes (> 55 μm) resulted at 7^{o}C (Fearman and Moltschaniwskyj 2010). Several studies have concluded that mature oocytes contain fatty yolk, mainly consisting of neutral lipids which are converted from glycogen. Meanwhile, lipids and a minor quantity of glycogen accumulate in the yolk of the oocytes and lead to the enlargement of oocyte diameter when developed during reproduction (De Zwann and Mathieu 1992; Chung 2007). Table 2 compares the mature oocytes size of the *C. grayanus* in East Sea, with the species belongs to the same family, that had been previously reported from temperate regions. The proportion of large mature oocytes declined from August to September; however, histological analysis demonstrated that most mussels were in spent and indifferent stages during August, and no females were in the spawning stage, suggesting that these oocytes were spent, with a short indifferent period between spawning and consecutive oogenesis.

Numerous studies have reported that carbohydrates are the main source of energy in marine bivalves, with almost 40–60 % found as glycogen (Beninger and Lucas 1984). Bivalves usually store carbohydrates as glycogen, and it can be used during gametogenesis. Nutrients are stored when gonadal activity is minimal, and the food source is abundant. The storage site varies with species, while in mussels the main storage tissue is the mantle, However, the usage of glycogen during gametogenesis will vary in mussels with the type of tissue (Gabbott 1975; Ngo et al. 2006).

In the present study, total carbohydrate levels fluctuated over the study period; the highest carbohydrate level was found in summer. The average carbohydrate content of different mussel species are compared with *C. grayanus* in Table 4. Relatively maximum carbohydrate levels were observed from late spring to the summer period (May to July), coinciding with the higher level of chlorophyll a in spring (Fig. 2). A similar observation was reported by Gabbott and Whittle (1986); there was a rapid increase in glycogen content in *M. edulis* in May to June at Anglesey, Wales, U. K. They concluded that net glycogen production takes place rapidly in the summer with an increased food source and glycogen synthetase enzyme. Most studies concluded that carbohydrate levels reached their maximum when the oocytes in the mature stage rapidly decrease during spawning time due to intense energy utilization by gonads and release of egg content (Zandee et al. 1980; Gabbott and Whittle 1986; Mondol et al. 2016). Further, glycogen will be converted to lipids, and the digestive gland contains higher amount of lipids, mainly triglycerides, just before spawning in mussels (Lubet et al. 1985). Contrary, there could not be seen decrease in total carbohydrate content during spawning of *C. grayanus*, which suggests the mussels accumulated energy for subsequent gametogenesis. It seems these mussels reabsorb their spent gonads

concurrently with developing storage tissue as reported in *M. galloprovincialis* in Spain (Villalba 1995). This stored energy during resting period substantially influence on reproductive effort, oocyte size and larval quality (Bayne 1976). Sudden reduction of total carbohydrate in late summer suggests inhibition of feeding due to high temperature (Bayne 1976). However, in autumn low temperature seems to promote feeding, increase energy reserves in *C. grayanus*, which is used for gametogenesis in winter. In the present study, the lowest carbohydrate level can be observed from winter to early spring (December to April). Similar observations were made in *M. edulis* in the winter; the food sources and glycogen synthetase are at their lowest and, therefore, the use of exogenous glucose and rapid transfer of glycogen to the mantle will be restricted. However, the energy requirement is increased due to maintenance as well. Reproduction finally leads to a breakdown of glycogen from mantle tissue (Gabbott and Whittle 1986).

Table 4 Comparison of average total carbohydrate of different Mytilus with *C. grayanus*. TCH- Total carbohydrate content.

Species	TCH (mg/ g DW)	Reference
M. galloprovincialis	115	Leontowicz et al. (2008)
M. galloprovincialis	178	Kopp et al. (2005)
M. edulis	298	Kopp et al. (2005)
M. edulis	131	Chi et al. (2012)
C. grayanus	217	Present study

Protein content of mussels varies with species, age, food, and other environmental factors. Accordingly, mussels rich in amino acids, including mainly glutamic acid, aspartic acid, glycine, alanine, proline, serine, lysine, and arginine, also showed little seasonal variation; a high proportion of amino acids occurred in the adductor muscle (Zandee et al. 1980; Chi et al. 2012; Kim et al. 2013). A low level of protein from fall to winter agrees with the finding of Zandee et al. (1980) which revealed that protein is used for both reproduction and energy production when food is scarce. The protein content gradually increases during developing stage, from November to February, seems that much of extra protein directly involved with gametogenesis in *C. grayanus*. A slight decrease of protein could be noticed in the spawning period in this study; however, as a consequence of gametogenesis, a rapid reduction in protein level was noticed in several studies (Zandee et al.1980; Kopp et al. 2005).

According the present study, the CI has not dropped considerably during the spawning period, although histology suggests that spawning commenced in May (Fig.3). The results suggest that there was a noticeable rise in energy reserves during spawning because of the chlorophyll a level increase in spring, which might be a reason for a higher CI in late spring and summer. Ruiz et al (1992) also reported that food availability and seasonal changes are the important cues for nutrient storage and energy allocation during growth thus influence on meat production in marine bivalves. The fluctuations in CI over this period were not associated with the reproductive cycle; however, in this study, it seems that the CI was related to stored energy reserves. Mussels utilized ingested energy directly or indirectly. *Mytilus edulis* uses both sources while *Modiolus barbatus* relies mainly on energy from ingested food which results in a higher CI (Bayne 1976; Mladineo et al. 2007). Zandee et al. (1980) also reported that

M. edulis showed the highest value of CI and a higher calorific value in summer which suggested that food value and food availability were somewhat higher. The sudden reduction in CI from September to October in the current study was not associated with the spawning activity of *C. grayanus*, because most mussels examined during this period were in indifferent and early developing stages (Fig 3). It appears that such reduction in CI during the indifferent reproductive period is associated with the use of stored energy (Yang et al. 2011). The complex interaction between temperature, food availability, and salinity, which is caused by seasonal changes, influences somatic growth and reproduction, thereby indirectly affecting CI (Zupan and Saric 2014). Similar observations in the Adriatic Sea (42º 54' N, 17º 35'E) reported that higher CI was recorded in mussels from April to July, which coincided with a high level of phytoplankton (Mladineo et al. 2007). We assume that *C. grayanus* were not develop storage tissue and gametes concurrently nevertheless subsequently, suggests environmental condition in east coast superior for mussel culture.

5. CONCLUSION

In summary, we first investigated annual gametogenesis and temporal changes in biochemical tissue compositions of *C. grayanus* on the east coast of Korea. Annual gametogenesis of *C. grayanus* was found to be closely linked to seasonal changes in SST. Gametogenesis commenced in September as the SST reached 19.5^{o}C. Spawning individuals were observed in late spring, in May, with a spawning percentage of 54.5% of their population. The total carbohydrate content of *C. grayanus* showed high seasonal variation while the protein content remained relatively the same during this study. The maximum monthly average of oocytes was observed in July, the minimum occurring in September. Currently, *Crenomytilus grayanus* at the study site is harvested without proper management practices, which may lead to a dramatic reduction of its population in the East Sea. Therefore, this study recommends a suspension of the harvesting of *C. grayanus* during the spawning period to protect and conserve spawning mussels, and to promote subsequent recruitment in the East Sea.

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