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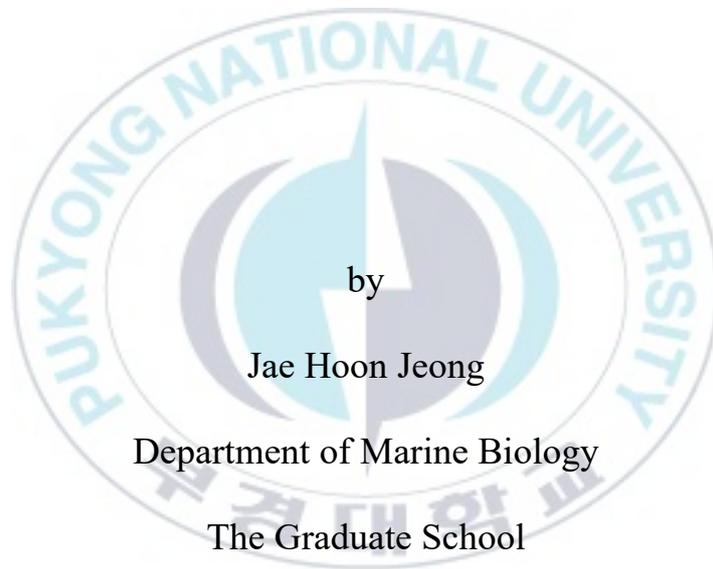
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Thesis for the Degree of Master of Science

Age determination and growth of *Cyclina sinensis* (Bivalvia, Veneridae) by external rings in Busan, Korea



by

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Department of Marine Biology

The Graduate School

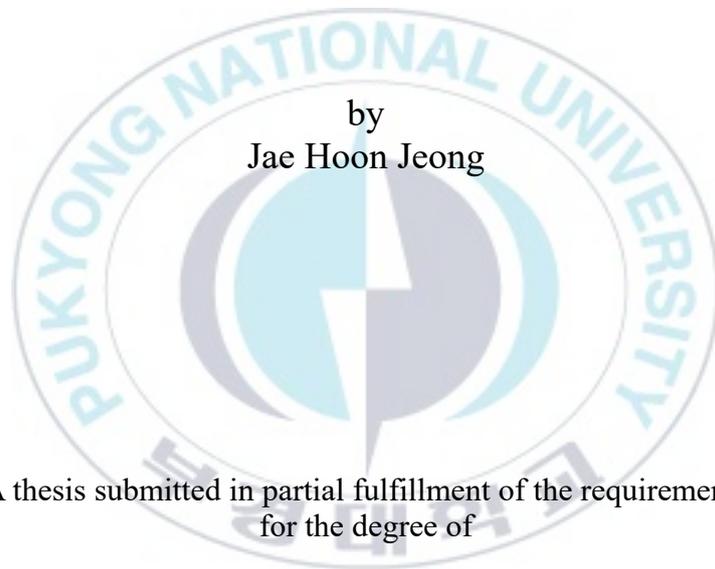
Pukyong National University

February 2021

Age determination and growth of *Cyclina sinensis* (Bivalvia, Veneridae) by external rings in Busan, Korea

(가무락 조개의 외부 성장륜을 통한
연령과 성장)

Advisor: Prof. Chul-Woong Oh



by
Jae Hoon Jeong

A thesis submitted in partial fulfillment of the requirements
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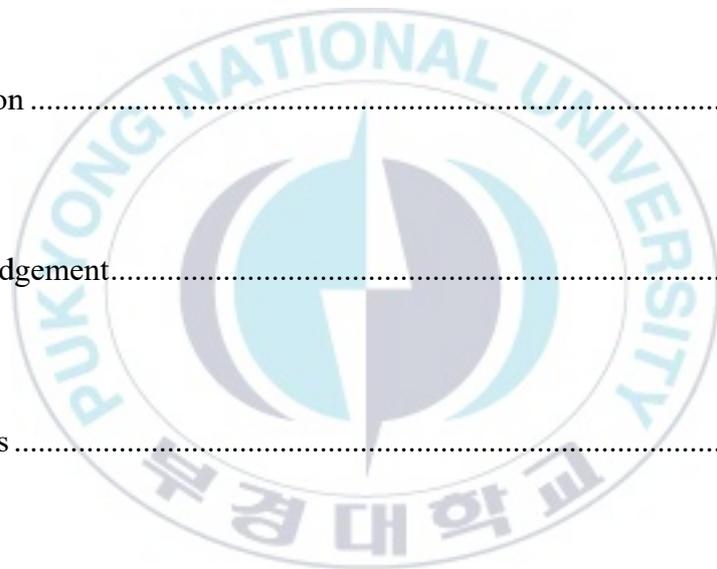
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Age determination and growth of *Cyclina sinensis* (Bivalvia, Veeridae) by external rings

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Abstracts

Age and growth of *Cyclina sinensis* were investigated during the period from August 2019 to September 2020 in Busan, Korea. The shell length range is 14.78 ~ 50.49 mm, shell height is 14.09 ~ 51.76 mm and shell width is 8.25 ~ 34.37 mm. The relationship between shell length (SL; mm) and shell height (SH; mm) was expressed by the equation $SH=1.0407SL-1.7958$ ($r^2=0.97$, $n=625$, $P<0.01$) and between SL and shell width (SW; mm) by the equation $SW=0.6884SL-2.3306$ ($r^2=0.92$, $n=625$, $P<0.01$). The relationship between SL and total weight (TW; g) was expressed by the following equation: $TW=0.00006SL^{3.4559}$ ($r^2=0.95$, $n=389$, $P<0.01$). We estimated the age of *C. sinensis* by reading the external rings on the shell. The age of this species ranged from 0 to 7 years. Based on external rings, the von Bertalanffy growth functions were expressed by the equation :

$$SL_t = 79.94(1 - e^{-0.114(t+0.947)})$$

$$TW_t = 188.28(1 - e^{-0.114(t+0.947)})^{3.4559}$$

1. Introduction

The venus clam *Cyclina sinensis* (Gmelin, 1971) is important edible bivalves in East Asian countries, including Korea, Japan and China (Chung et al., 2007). In Korea, this species is mainly found in silty clay in the intertidal coastal waters of Korea (Kwon et al., 1993). The main spawning season is from July to August, when the water temperature is high, and the growth rate is low, so three to four years after hatching are needed to have food value.

Domestic annual production of *C. sinensis* has continued to decline after recording 2,424 M/T in 2010, and showed low production, recording 806 M/T in 2018. As a result, the amount imported over a decade accounts for about 70% of the consumption (Statistics Korea, 2018).

At proximate composition in the Muscle and viscera of five venerid clams (Bivalvia) from Southern Coast of Korea (Yoon et al., 2008), the crude protein content of the hemiplegia was $9.0 \pm 0.26\%$ higher in the muscle area, and $2.01 \pm 0.19\%$ higher in the viscera region of the cell. Based on these studies, it is judged that the food value and consumption should be increased through the search for ways to utilize them in a nutritional way. Age validation is important in ageing

commercial species (Beamish and MacFarlane, 1983). Panfili et al. (2002) stated that theoretically, validation is required for every population of any given species because there may be important differences between populations. Age structure and growth data are needed for fisheries' models that are used to explain the dynamics of a fish population or fishery resource.

Ring formation has been associated with factors that probably affect the metabolic processes involved in growth, such as extrinsic environmental conditions (temperature) or biological conditions (spawning) (Ropes, 1987).

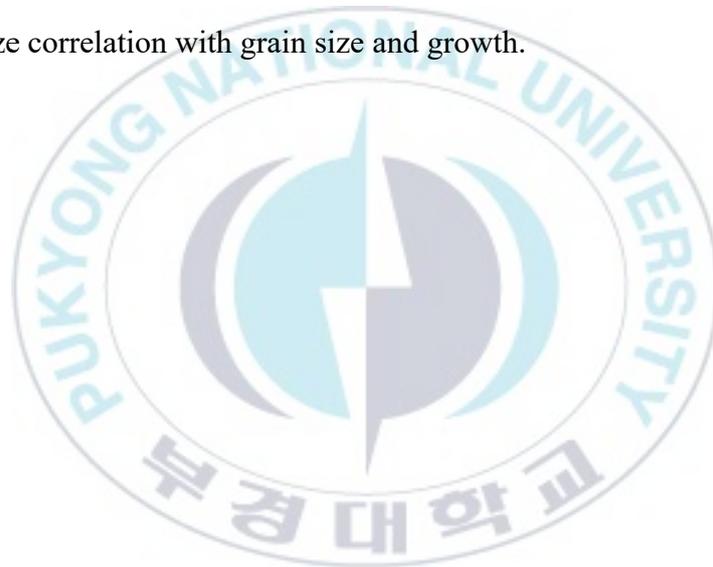
Currently, the production of *Cyclina sinensis* (Gmelin, 1971) have decreased, but no previous research has been conducted about reason of the decrease in the production of *C. sinensis*, and the ecological research of *C. sinensis* is also lacked. There is also no study of distribution and age growth according to recruitment.

The estuary of the Nakdong River is the largest delta on the Korean Peninsula, especially at the front of the delta's end, with various large and small sages formed to match the coastline, and a wide mudflat is distributed between these barrier island and the end of the delta. The mudflats in this area are composed of alluvial layers of 50 to 60 meters thick, and are largely composed of the lower gravel or gravel-sand mixture, the central silt clay layer or the upper sand layer, and the upper sand layer or the silt-sand layer (Oh, 1992). But there is no research about correlation

with grain size and growth.

Previous study are seasonal changes in biochemical components of the adductor Muscle and visceral mass tissues in the female *C. sinensis*, in relation to gonad developmental phases (Chung et al., 2004), Research on seasonal reproductive cycles and genetic morphological changes in China is conducted. Therefore, in this study, we would like to research a foundation for population growth and stable production through the overall ecological information survey of *C. sinensis*.

The aims of this paper are to reveal biological characteristics and age and growth and to analyze correlation with grain size and growth.

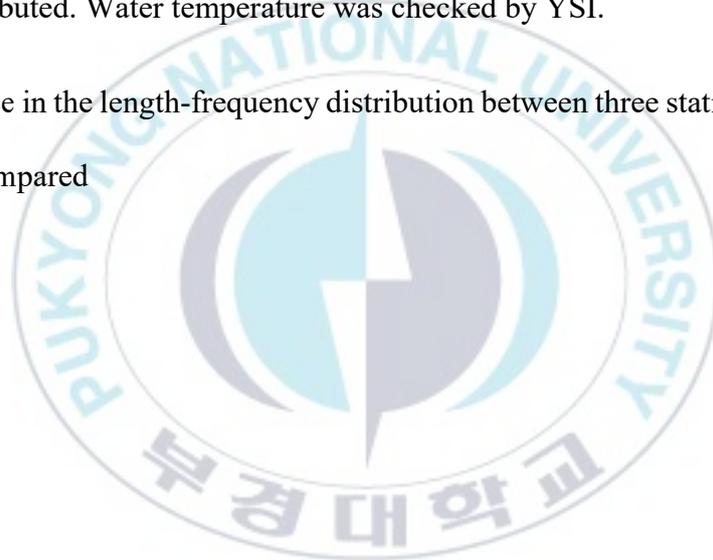


2. Materials and Methods

2-1. Sample collection

Cyclina sinensis (Gmelin, 1971) used in the study were collected using shovel in Busan, Korea and taken from August 2019 to September 2020 (Fig. 1). The sampling station was located at a depth of 2 m in an area where *C. sinensis* was mainly distributed. Water temperature was checked by YSI.

Difference in the length-frequency distribution between three stations (St.1, St.2, St.3) was compared



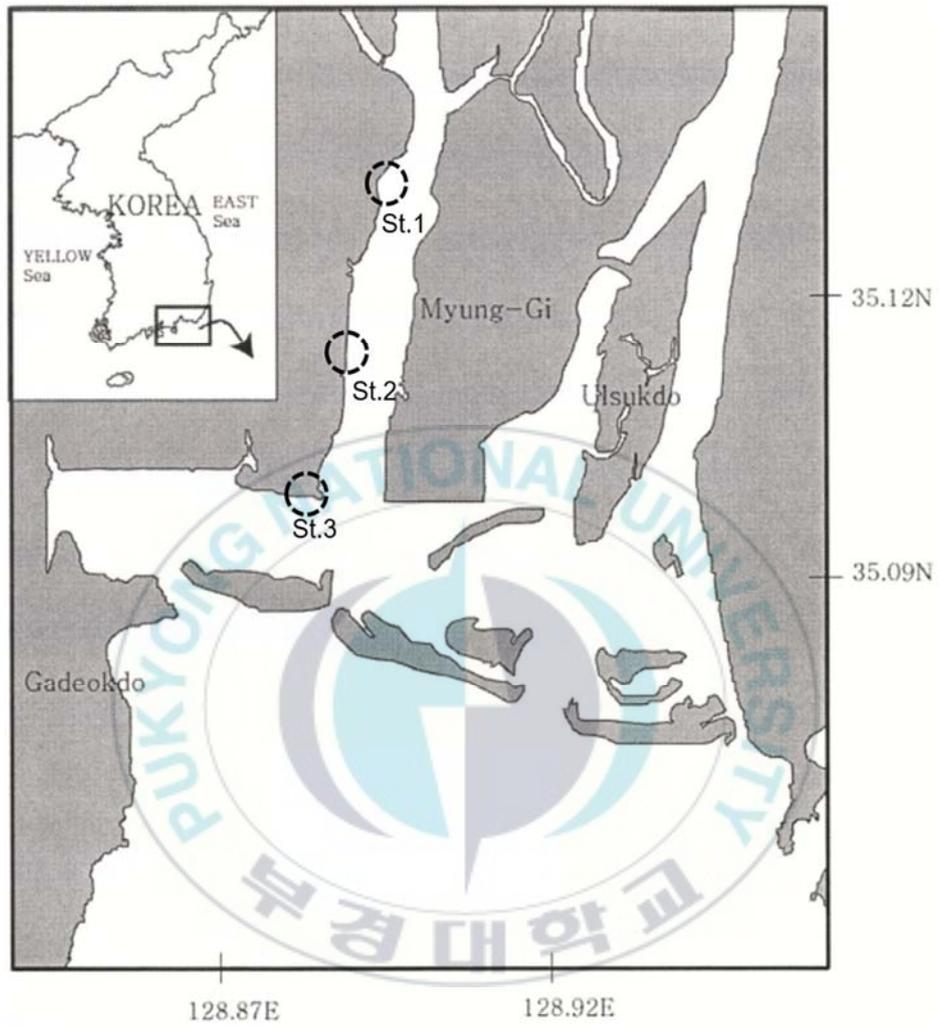


Figure 1. Map showing sampling area of *Cyclina sinensis* in Busan, Korea.

2-2. Morphometric relationship

The shell length (SL), shell height (SH) and shell width (SW) of each specimen was measured to the nearest 0.01mm using the Vernier caliper. Conversions among length measurements can generally be achieved with simple linear regressions models.

Total weight (TW) was checked with an electronic analytical balance to the nearest 0.01g. The relationship between SL and TW was determined by function using the equation :

$$TW = aSL^b$$

Where a and b are constant, TW is the total weight (g) and SL the length (cm). A specific weight-length relationship was established for the total sample.

2-3. Age determination (External rings)

The age estimation allows us to know the dynamics of fishery resources and predict the direction of fishing.

After dipping the shell in 32% hydrochloric acid for 20 seconds, the corroded

shell is considered to be the part where only the cornea is removed and the ridge layer is visible (transparent band), and projected into the natural light for visual observation. (Lee and Kim, 2017)

The age was determined by reading the boundary between the opaque zone and the transparent one shown in the shell. To increase the accuracy of each reading, records were double checked. First, visual discrimination was conducted, and two age assessments were carried out using hydrochloric acid for worn or poorly visible shellfish in the sample, and then using a lighting device that transmits light to the shell.

In this survey, we observe objects with one ring in the shell, two ring in the shell, In order to find out the timing of the leap gate formation and the number of leap gate formation due to seasonal periodicity, a Marginal Index (MI) is obtained, and based on this, the timing of the marginal formation is analyzed.

2-4. Growth parameter

Marginal index (MI) analysis was used to validate the annual growth pattern increment deposition (lai et al., 1996) that is calculated by the equation :

$$MI = \frac{R-r_n}{r_n-r_{n-1}}$$

where R is the Shell length, r_n is the shell length at the time of the n th transparent zone mark (Fig. 2).

Fatness index for estimate spawning period (Lee et al., 2013) is calculated by the equation

$$Fatness = \frac{MW}{SL^3} \times 10^3$$

Growth parameters were estimated using von Bertalanffy growth (VBG) by the Walford method and non-linear regression for the *C. sinensis* by the equation:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

$$TW_t = TW_\infty(1 - e^{-K(t-t_0)})^b$$

where L_t is the length at age t , L_∞ the asymptotic length, K the coefficient of growth, t_0 the theoretical age when predicted mean length zero, b is constant of correlation equation with Shell length and total weight.

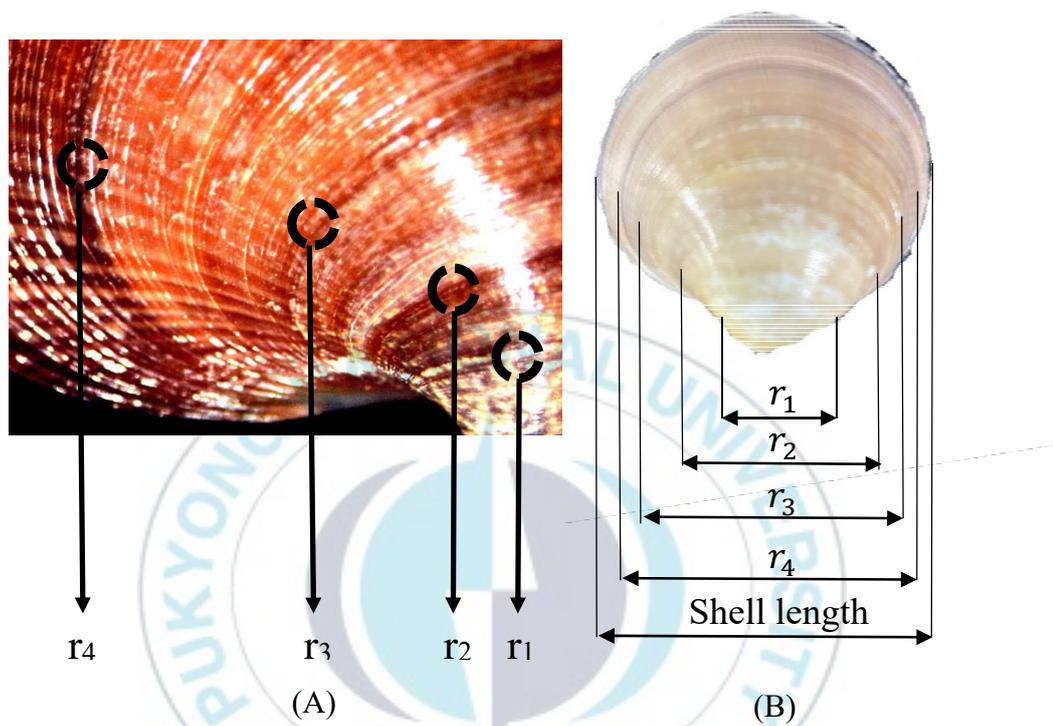


Figure 2. A, The ring observed under the microscope. B, Shell length (SL) and ring diameters of clam *Cyclina sinensis*.

For comparison with previous study of other venerid species, the growth performance index (ϕ) was calculated in each case following the formula (Munro and Pauly, 1983).

$$\text{Growth performance Index } (\phi) = 2 \log L_{\infty} + \log k$$

2-5. Multiple analysis between sediment type and biological factors

We conducted sampling to see the correlation between seasonal shell length and grain sizes in two places 1.5 km away from the sampling site every month. Seasonally, winter sample was collected in December 2019, Spring sample in April 2020, Summer sample in July 2020 and Fall in September 2020. To determine the native sediment grain size and compare to sediments difference of each station, replicate sediment samples were taken from each station at the time of collection, Grain size distribution (the proportion of mud, sand and gravel) was determined by oven drying each sample at 100°C overnight and washing each subsample through stacked sieves with meshes of 2 mm and 63 μm sieves, to provide the proportion of gravel (>2 mm), sand (63 μm -2 mm), and mud(<62 μm). The fraction retained on each sieve was dried and reweight. The dry weights of each fraction were then

expressed as percentages of the total dry weight (Tara Anderson et al., 2019).

Multivariate ordination (nonmetric multidimensional scaling: n MDS) was used to examine similarities in particle size analysis (Percentage of gravel, sand, silt, clay, mud, Mz, sort, skew, kurt) and biological factors (water temperature, average of SL, number of species) with site and season. For multivariate analysis, data were transformed into a four-root in order to minimize the error between grain size and biological factors and the difference was tested for significance using ANOSIM (Analysis of similarities) (Oh et al., 2009). The stress value suggested in the multidimensional scaling method is value indicating the presence or absence of separation between groups. In general, when <0.2 , it means that there is a significant difference between groups, and the value of the stress value increases as the amount of sample and the displacement between samples increase is done. (Clarke, 1993). ANOSIM analysis can be analyzed only when the stress value is <0.2 , and in ANOSIM analysis, the R-value is >0.5 , which means that there are significant differences between groups. (Clarke and Gorley, 2001). All statistical analysis was performed using PRIMER (Plymouth Routines In Multivariate Ecological Research; Version 5.2.4).

3. Results

3-1 Morphometric relationship

The samples of *Cyclina sinensis* ranged from 14.78 to 50.49mm in shell length (SL) and from 0.59 to 46.2g in total weight. Mean total length and weight was 34.63mm and 14.11g, respectively.

The relationship between shell length (SL; mm) and shell height (SH; mm) was expressed by the equation $SH=1.0407SL-1.7958$ ($r^2=0.97$, $n=625$, $P<0.01$) and between SL and shell width (SW; mm) by the equation $SW=0.6884SL-2.3306$ ($r^2=0.92$, $n=625$, $P<0.01$) (Fig. 1) The relationship between SL and total weight (TW) was expressed by the following equation: $TW=0.0006SL^{3.4559}$ ($r^2=0.95$, $n=389$, $P<0.01$) (Fig. 2). We estimated the age of *C. sinensis* by reading the external rings on the shell.

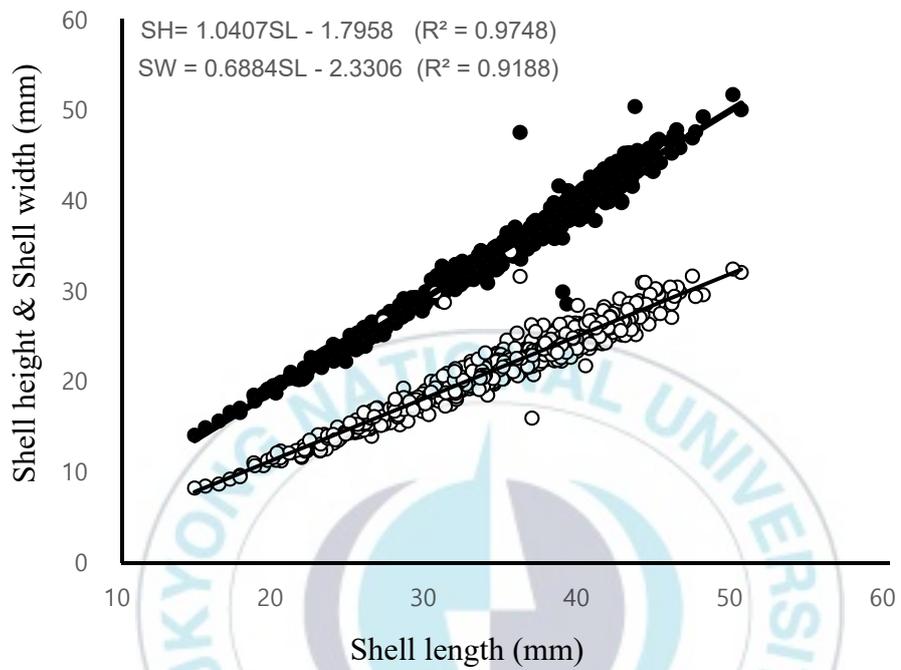


Figure 3. Relationship between shell height (SH) and shell width (SW) and shell length (SL) of clam *Cyclina sinensis*

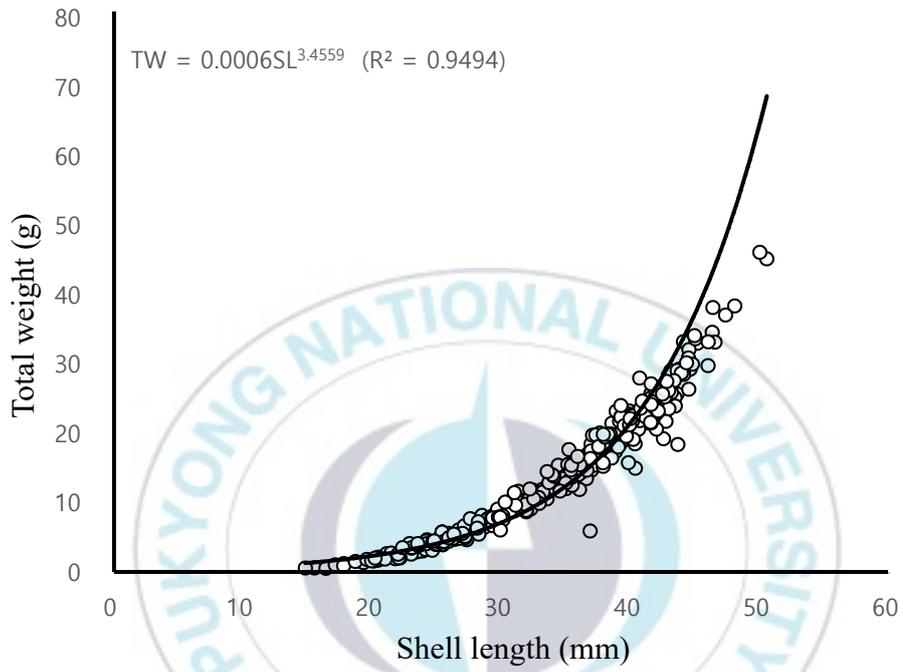


Figure 4. Relationship between shell length (SL) and total weight (TW) of *Cyclina sinensis*

3-2 Ring formation

Relationship between shell length and ring diameter was observed for the external rings taken from the specimen. The correspondence between shell length and radius was used to see if radius, the boundary of transitioning from opaque to transparent in shell, actually represents age. A study of the interaction relationship between the each group's radius and diameter showed that they were distributed around the regression line, with each ring diameter showing a relatively distinct correspondence.



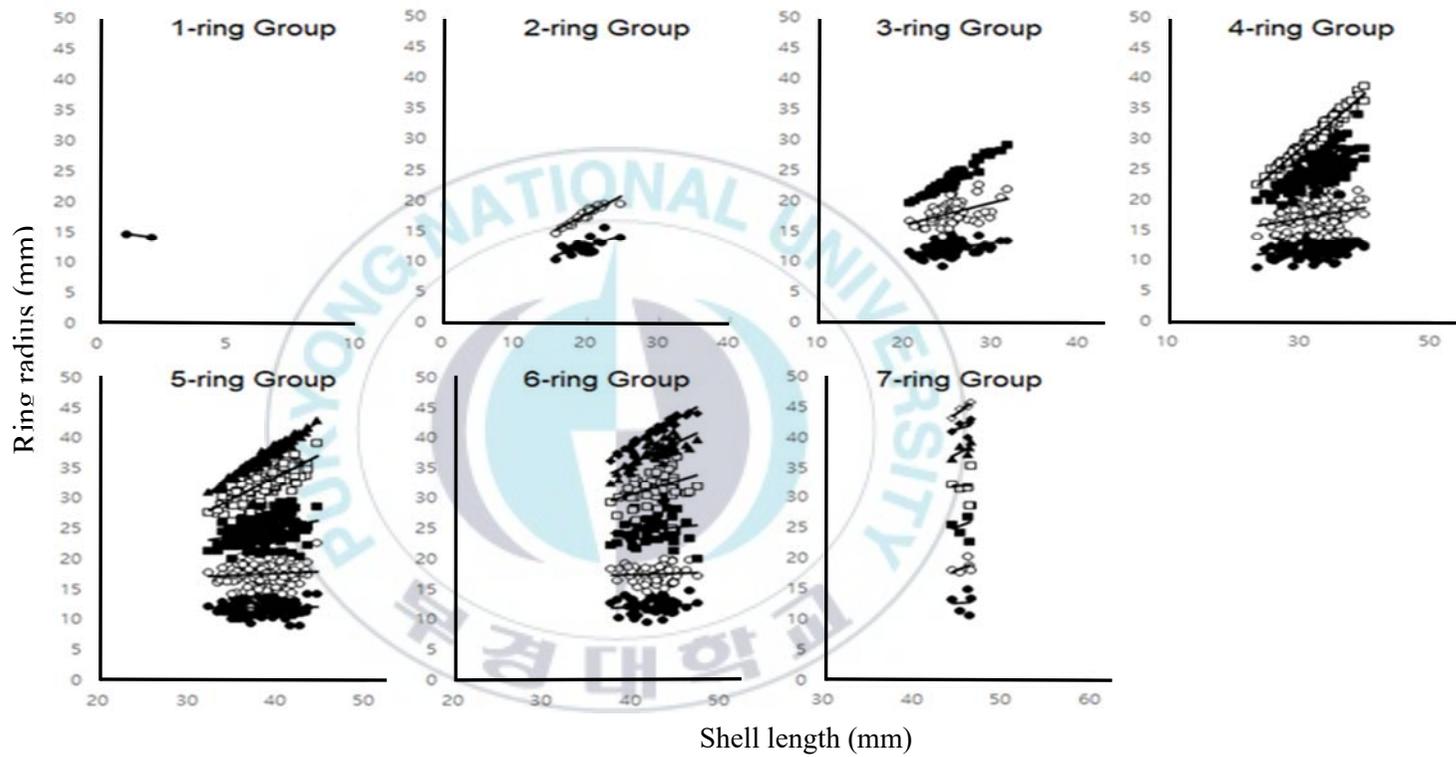


Figure 5. Relationship between shell length and ring radius of *Cyclina sinensis* in Busan, Korea

3-3 Marginal index (MI)

The ring diameter has been shown to be available as an age type. However, since the ring diameter does not necessarily represent an age, it was necessary to determine when and how many times a year the ring diameter was formed.

The mean monthly marginal index (MI) was calculated for the specimens each month to examine the relative growth patterns of the shell during the research period (Kim et al., 2016). The MI of the shell peaked in August 2019 decreased gradually from September to February, and reach the minimum at the February (Fig. 5).



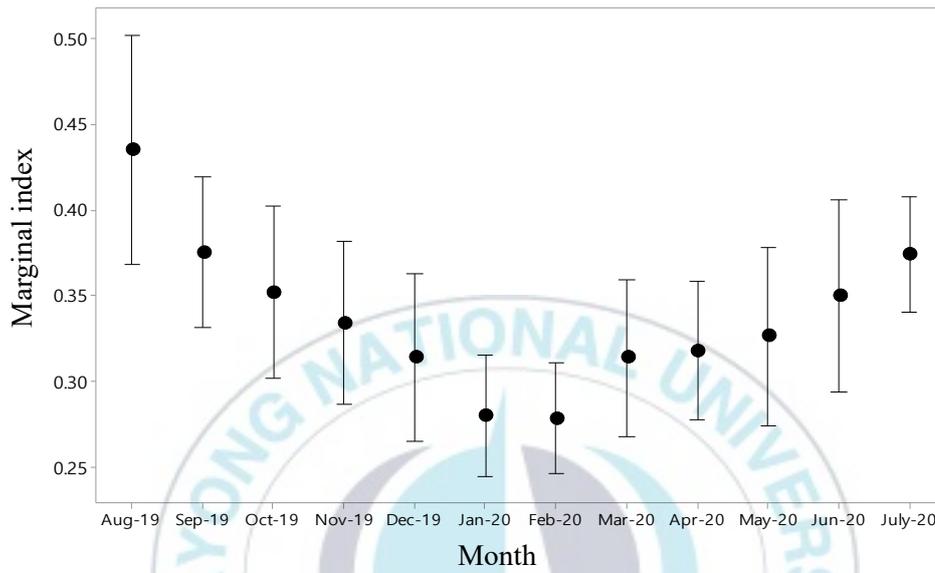


Figure 6. Monthly change of shell marginal indices clam *Cyclina sinensis* from August 2019 to July 2020

3-4 Fatness

The variation in fatness using meat yield to estimate the spawning period of shellfish was shown in the Fig. 6. The Fatness index were peaked in February 2020 and decreased gradually from May to July, and reach the minimum in August and November.



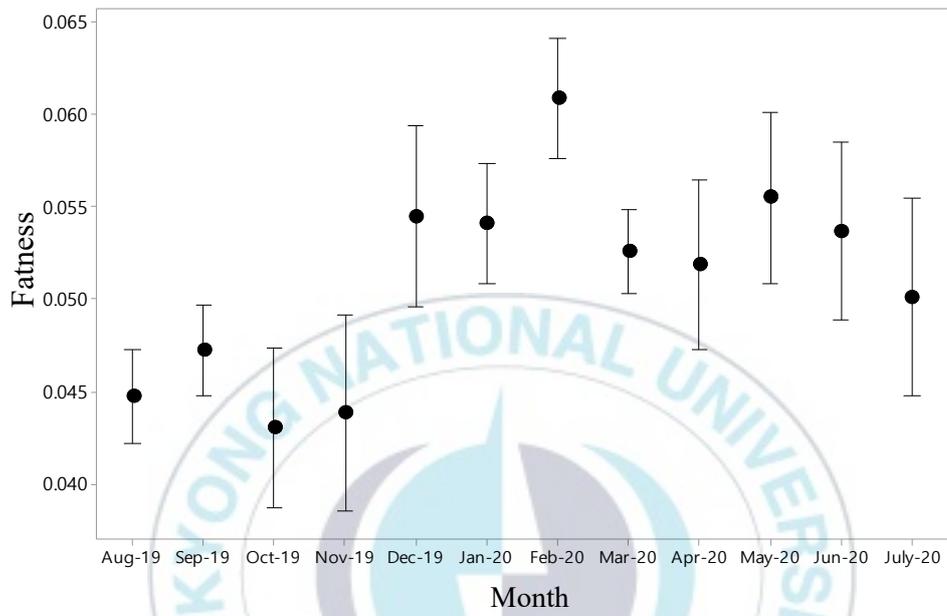


Figure 7. Monthly change in the fatness of clam *Cyclina sinensis* from August 2019 to July 2020

3-5. Monthly frequency distribution of shell length & age composition

The maximum shell length is found at March and minimum length at January (Fig. 6). The age of *C. sinensis* collected ranged from 1 to 7 years in external rings and the age group 5 and 6 were the dominant groups (Table 1).



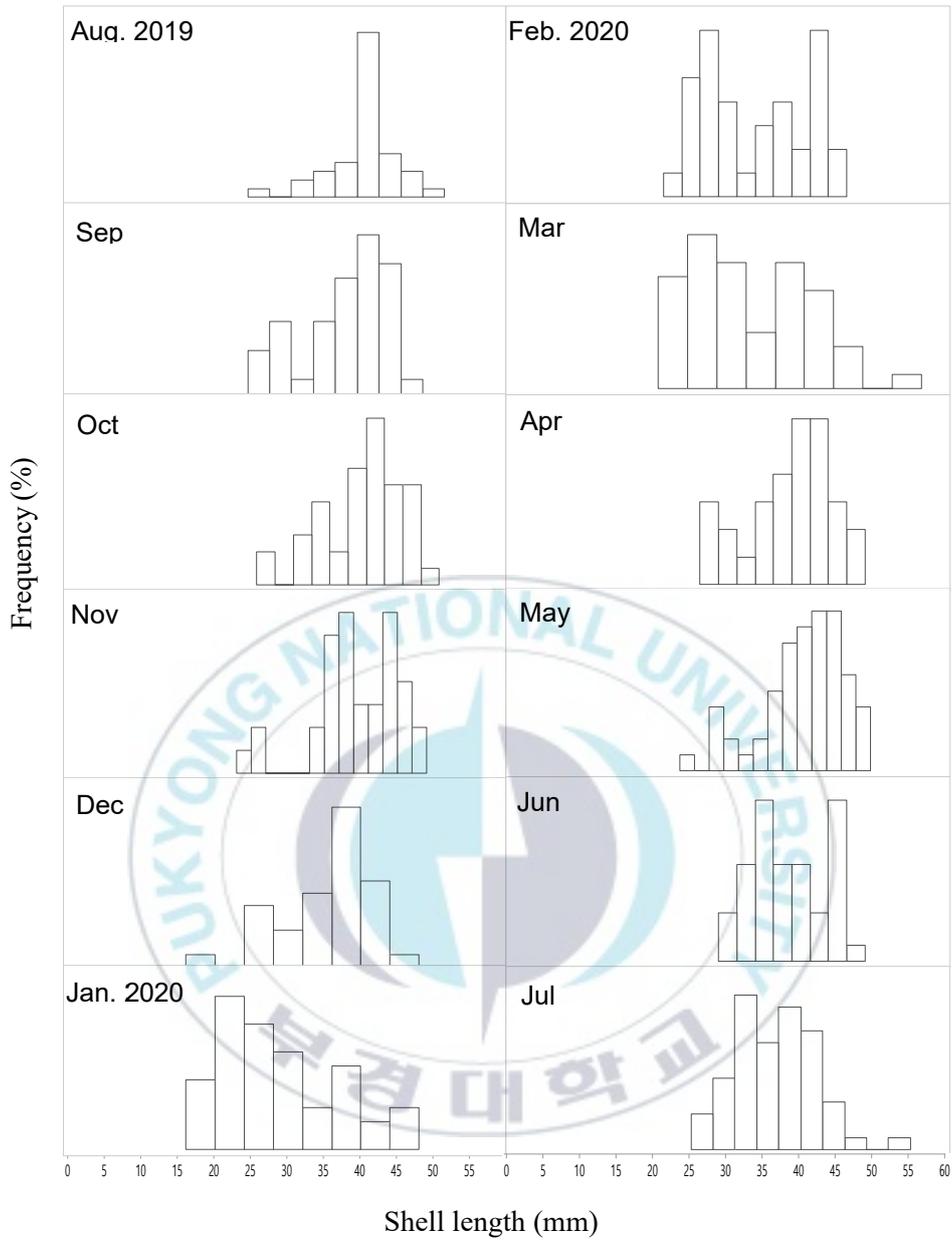


Figure 8. Monthly frequency distribution of the shell length of *Cyclina sinensis* on the Busan, Korea

3-6. Growth parameter

The von Bertalanffy growth (VBG) equation was estimated from the back-calculation of the mean shell length at the age of *C. sinensis*. Age ranged from 1 year to 7 years (Table. 1).

The shell of r_1 was 12.51 mm r_2 : 17.79 mm, r_3 : 24.72 mm, r_4 : 31.75 mm, r_5 : 37.49 mm r_6 : 41.41 mm r_7 : 44.69 mm (Table. 1). The estimated VBG parameter asymptotic length (SL_∞) was calculated with the nonlinear regression, and was 79.94 mm for the external rings. The growth equation was estimated using the growth parameters obtained by the Walford (1946) method. The growth parameter(k) is 0.071/year, When shell length was 0, theoretical age(t_0) is -0.947 year. The von Bertalanffy growth functions were expressed by equation (Fig. 9):

$$SL_t = 79.94(1 - e^{-0.114(t+0.947)})$$

In addition, the total weight (TW) of equation was estimated from the back-calculation of the mean diameter (Table. 2), the weight of r_1 was 1.06 g, r_2 : 1.90 g, r_3 : 4.07g, r_4 : 8.81 g, r_5 : 16.54 g, r_6 : 24.44 g, r_7 : 36.47 g and total weight and age equation (Fig. 10) :

$$TW_t = 188.28(1 - e^{-0.114(t+0.947)})^{3.4559}$$

Table 1. Estimated shell length at the time of each ring formation based on the relationship between shell length and total weight of clam *Cyclina sinensis*

Item	Ring group	No.	Ring diameter							
			r_1	r_2	r_3	r_4	r_5	r_7	r_7	
Shell length (mm)	1	1	14.21							
	2	19	12.37	17.64						
	3	63	12.16	18.06	23.56					
	4	141	11.89	17.49	24.82	30.75				
	5	141	11.99	17.46	27.72	32.55	36.88			
	6	50	12.26	17.52	24.89	31.79	37.60	41.04		
	7	5	12.68	18.58	25.61	31.89	37.98	41.77	44.69	
		420	12.51	17.79	24.72	31.75	37.49	41.41	44.69	

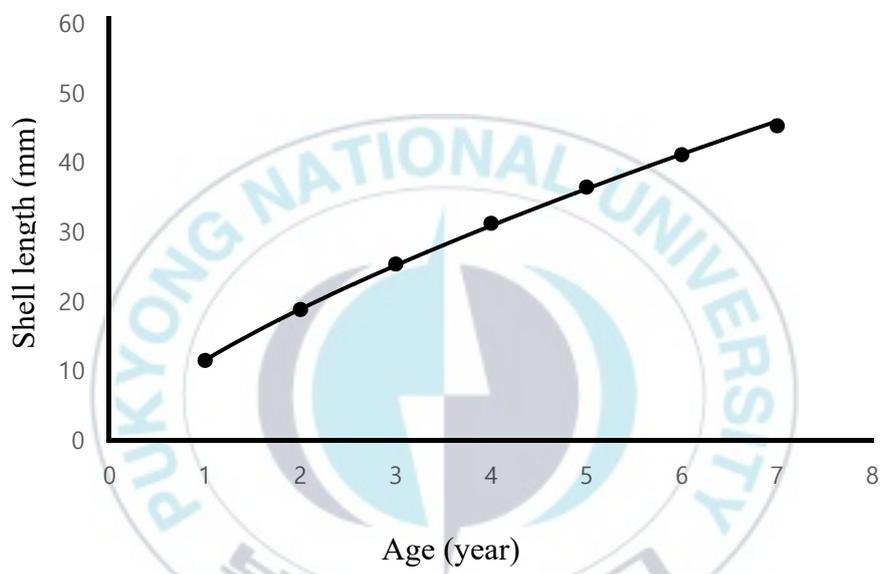


Figure 9. Von Bertalanffy growth curves of *Cyclina sinensis* estimated by nonlinear regression by external rings in Busan, Korea

Table 2. Back-calculated total weight at estimated age of *Cyclina sinensis*

Item	Ring group	Ring diameter						
		r_1	r_2	r_3	r_4	r_5	r_6	r_7
Total weight (t)	1	1.28						
	2	1.05	1.87					
	3	1.02	1.95	3.57				
	4	0.99	1.83	4.11	7.88			
	5	1.00	1.83	4.06	9.60	15.46		
	6	1.03	1.84	4.14	8.84	16.72	24.42	
	7	1.08	2.07	4.48	8.93	17.44	23.46	36.72
			1.06	1.90	4.07	8.81	16.54	25.44

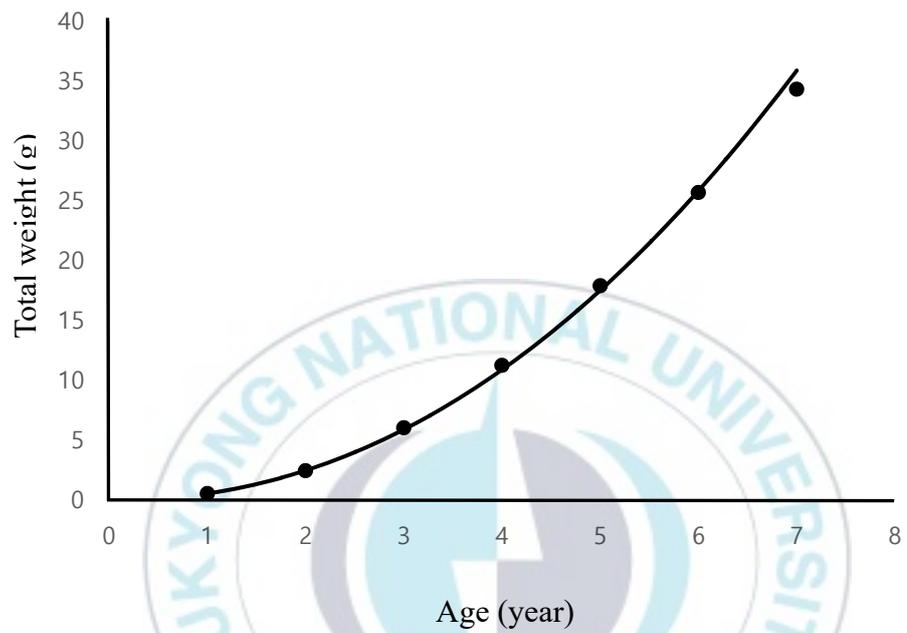


Figure 10. Total weight-based von Bertalanffy's growth curve in *Cyclina sinensis*.

3-7. Multiple analysis between sediment type and biological factors

There is significant difference in mean shell length by station ($F=32.77$, $df=2$, $P < 0.001$). Mean shell in St.1 is 34.624 ± 4.411 , St2 is 34.192 ± 5.430 and St.3 is 29.605 ± 8.167 . Younger specimen was found at St.3. A significant difference in length by season was found ($F=6.47$, $df=3$, $P < 0.001$). Mean shell of winter (September 2019) is 30.182 ± 6.816 , spring (April 2020) is 32.386 ± 6.685 , summer (July 2020) is 32.526 ± 6.875 and fall (September 2020) is 33.512 ± 6.816 . Lowest shell length is winter and maximum is fall. So small specimens appeared in winter, large specimens appeared in summer and early fall.

Native sediments from the Nakdong river were dominated by sand (>92%), with small quantities of Gravel (>0.1%), silt (>5.2%), clay (>2.7%) and mud (>7.9%). The mean percentage of st.1 are gravel (0.2%), sand (92.9%), silt (4.3%), clay (2.6%) and mud (6.9%). St. 2 are Gravel (0%), sand (90.6%), silt (4.3%), clay (2.6%), mud (9.4%). St.3 are gravel (0%), sand (92.5%), silt (5.2%), clay (2.3%), mud (7.5%). Triangular diagram of grain size are show at Figure 11.

The results of applying the multidimensional scale method for cluster analysis showed that clusters were formed for St.3 but were not formed for St.1 and St.2 (Fig. 12). In the case of seasonal cluster analysis, there were not formed except in winter.

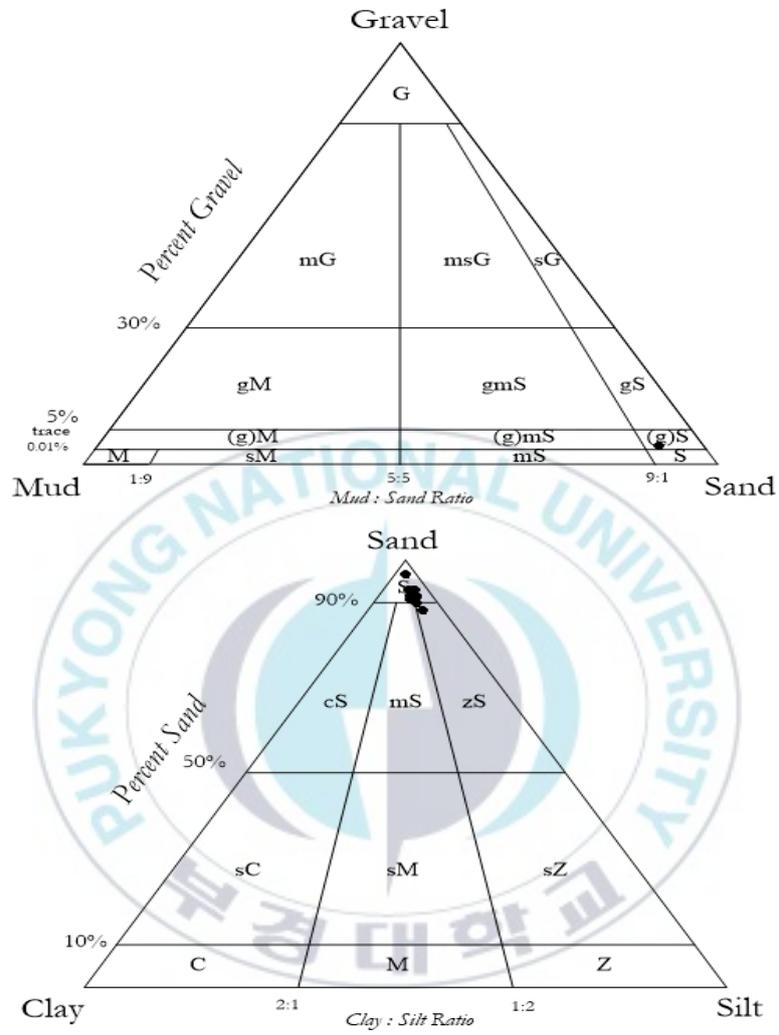


Figure 11. Triangular diagram of grain size at Busan, Korea.

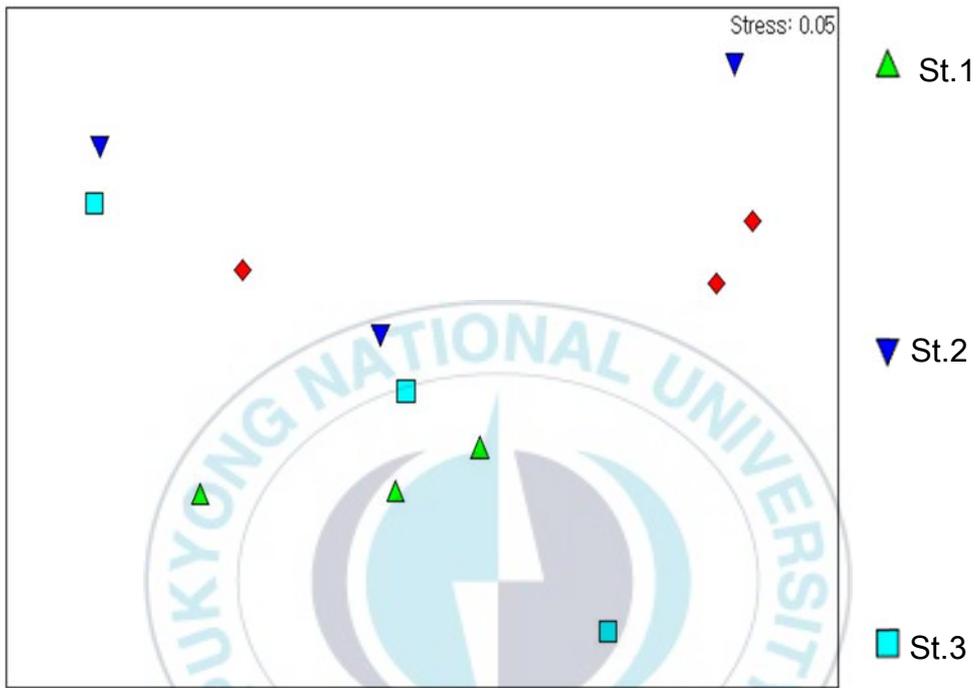


Figure 12. Non-metric MDS ordination plots in site

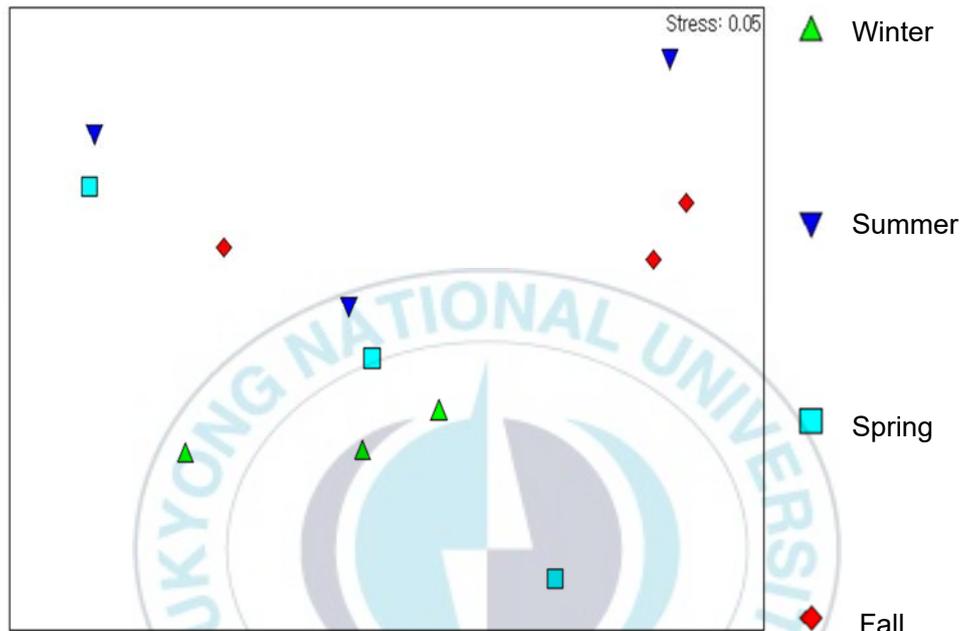


Figure 13. Non-metric MDS ordination plots in season

ANOSIM further supported the MDS results, showing significant differences among site (Table. 3). The results was divided St.3 and St.1, St.2 but there were no significant differences at season (Table. 4)



Table 3. Results of ANOSIM one-way analysis (Site)

Groups	R Statistic	Significance Level
St.1, St.2	0.063	0.20
St.1, St.3	0.865	0.029
St.2, St.3	0.51	0.029

Table 4. Results of ANOSIM one-way analysis (Season)

Groups	R Statistic	Significance Level
Winter, Summer	0.185	0.3
Winter, Spring	0.111	0.5
Winter, Fall	0.444	0.2
Summer, Spring	-0.037	0.5
Summer, Fall	-0.111	0.5
Spring, Fall	0	0.5

4. Discussion

In this study age ranged 1 to 7 years. Shell ranged from 1 to 7 ring group in *Cyclina sinensis* were 10.16-14.21 mm, 14.65-19.56 mm, 19.67-29.17 mm, 22.54-38.69 mm, 31.02-43.02 mm, 36.21-44.19 mm, and 43.25-45.39 mm, respectively. Largest SL, SH, SW were 50.49 mm, 51.76 mm, 34.37 mm. Compared with previous studies on the shell growth of *C. sinensis* (Lee and Song, 1973), 1 ring group to 5 ring group of *C. sinensis* were 12–17 mm, 22–27 mm, 25–31mm, 29 – 38 mm, and 36-42 mm, respectively. The largest shell length (SL), shell height (SH) and shell width (SW) were 42 mm, 44 mm, and 25 mm, respectively. The shell length in previous study and present study is similar in 1 to 3 age group but 4 to 5 group is bigger than this study. Further research is needed to determine whether this phenomenon is due to the cause of the changes in the quantity and quality of food sources and changes in the surrounding marine ecosystems such as rising waterways in summer and the opening of floodgates in the Nakdong river.

In this study, alive specimens at August 2020 and September 2020 were not observed. This could be affected by two times typhoon. First one occurred at 26 August and second on 02 September. The natural or anthropogenic events that deposit sediments with similar grain sizes to those already present would have limited direct impacts on local populations. Changes in the grain size distributions

and composition of natural deposited sediments are also known to burrowing ability and survival of infaunal species (Anderson et al., 2004). Higher mortality would be expected at thicker deposits of sediments. Where deposited sediment differs in texture from the sediment, or are comprised of terrestrial muds, or where freshwater come in, changes in loss of critical species and community structure (Tara Anderson et al., 2019). More frequent storms, associated with long-term climate change, are also likely to increase the risk of burial with non-native sediments accompanied by physical disturbance (Tara Anderson et al., 2019). As we saw in the MDS results, there were no significant difference of between season, but in St.1, it its expected that there will be many inflows of fresh water as it is located relatively upstream. Further research will be needed through additional sampling by increasing the number of species.

Based on histological and cytological observations of its gonad, the percentage of sexually mature individuals of 25.1 to 30.0 mm in shell length was over 50%, and that over 35.1 mm 100% (Chung et al., 2007). In this study individuals of over 30 mm in August and September were 92% and 79%, respectively. Accordingly, most individuals in this study area seem to be able to reach maturity by September (Chung et al., 2007). This means that larger individuals can reach maturity earlier than smaller individuals.

The ring has been shown to be available as an age character. However, since the ring does not necessarily represent an age, it is necessary to determine when and how many times a year the ring was formed. This study showed that the rings of *C. sinensis* are formed once a year, from January to February. Estimated changes in fatness are the highest in August 2019 and July 2020, so the spawning period is likely to be between July and August. It has been confirmed that the first ring of *C. sinensis* is formed in about 0.5 years and once a year, starting from July to August. *Meretrix lusoria* and *Ruditapes philippinarum* ring formed in February but *Ruditapes philippinarum*, *Gomphina (Macridiscus) veneriformis* ring formed in July and August. Spawning period of *C. sinensis* were consistent with ring formation period.

Comparing with other species, the fatness value for *Gomphina (Macridiscus) veneriformis* was lower in July and August, which indicated the main spawning season. *Meretrix lusoria* formed ring between July and August. From these results, it is concluded that the main spawning period for most venerid species can fall into July-August.

The asymptotic length (SL_{∞}) of *C. sinensis* calculated by the von Bertalanffy growth curves was 79.64 mm (non-linear regression method). Other species in the same family *S. purpuratus* of the maximum shell length (SL_{∞}) was 126.16 mm (Lee

et al., 2018), *M. lusoria* 104.94 mm (Ryu et al., 2006), *R. philippinarum* was 51.01 mm (Yoon et al., 2011) and *G. veneriformis* 70.80 mm (Table. 3) (Fig. 14) (Kim et al., 2014). Growth performance index (ϕ') of *C. sinensis* is 2.86. Compare with other species, maximum of growth performance index (ϕ') is *S.purpuratus* (3.51) and minimum species is *R. philippinarum* (2.66). *R. philippinarum* growth performance index is similar to *C. sinensis*. The bivalve species show reduced growth in winter as a result of declining temperatures or decreased food abundance, disturbance, predator attacks, and detrimental algal blooms (Richardson, 1993).

Age structure and growth have to provide for fisheries models to assess the health of fishery resource or to correctly analyze the dynamics of a fish population. In general, growth increments of marine animals are estimated annually from hard parts, such as scales, otoliths, or shells (Kim et al., 2016). Bivalve molluscs have been aged by examining the external valve surfaces for dark growth “rings” or “bands” that form as an annual periodic event (Feder and Paul, 1974).

The common method observing with lighting device is efficient in terms of time and economy since no additional procedure is required. However, thick shells were hard to observe. In this case, there is a method of cutting and dissolving with hydrochloric acid or dyeing the shell. Other methods are counting of annual growth marks or rings visible in the microstructure of shell sections (Richardson et al.,

Table 5. Growth parameters (L_{∞} , K , t_0) and growth performance (ϕ')

Species	Regions	Parameter				Reference
		L_{∞} (mm)	K	t_0	ϕ'	
<i>Saxidomus purpuratus</i>	Jinhae Bay	126.16	0.203	-0.52	3.51	Lee et al., 2018
<i>Meretrix lusoria</i>	Tidal flat of Simpo, Puan-gun	104.9	0.2235	-0.77	3.39	Ryu et al., 2006
<i>Ruditapes philippinarum</i>	Tidal flat of Goheung	51.01	0.1738	-0.17	2.66	Yoon et al., 2011
<i>Gomphina (Macridiscus) veneriformis</i>	Wonsan coast of East Sea	70.80	0.217	-0.367	3.04	Kim et al., 2014
<i>Cyclina sinensis</i> (This study)	Busan	79.93	0.114	-0.947	2.86	

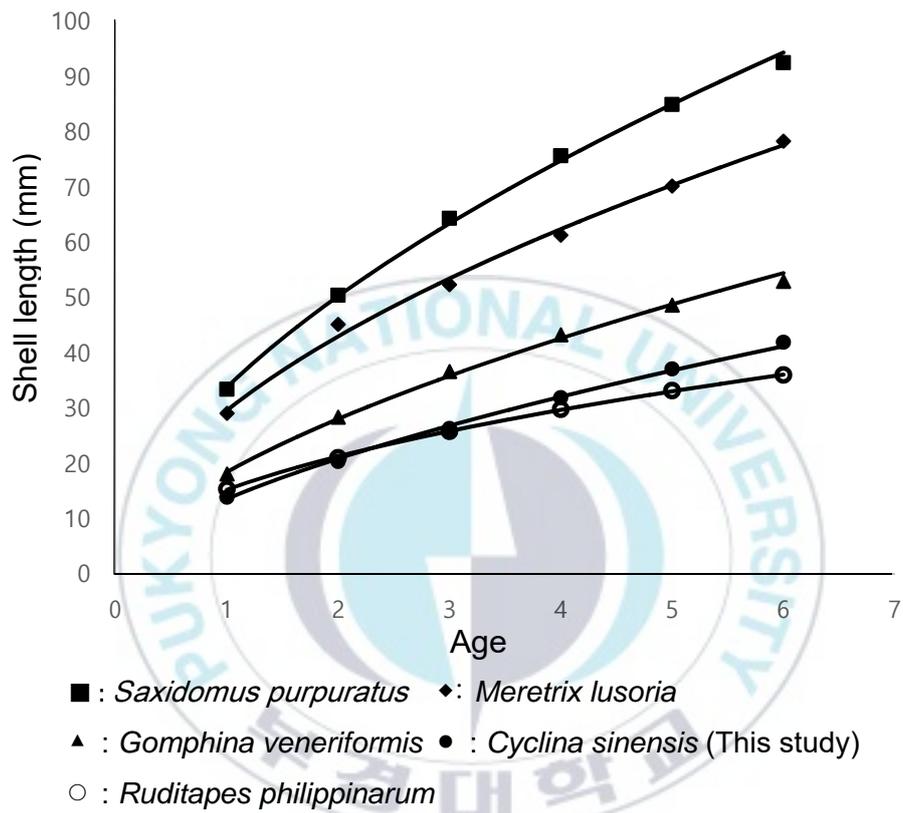
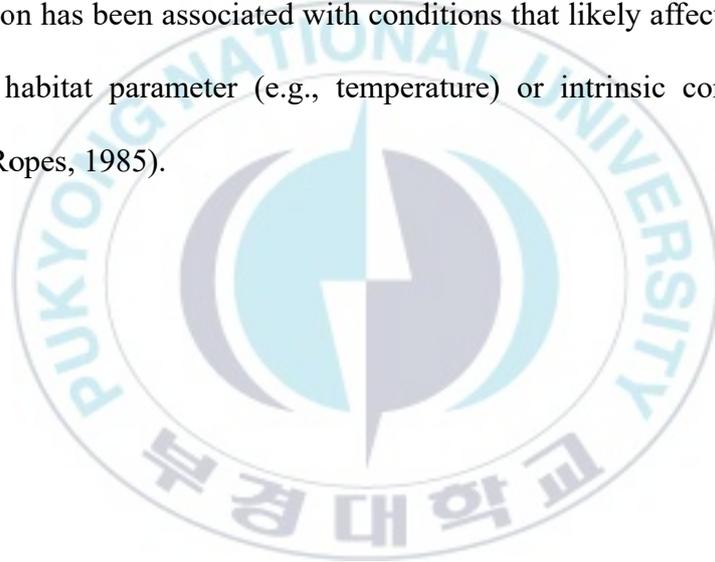


Figure 14. Von bertalanffy growth curves of Venerid species.

1990), mark and recapture experiments (Gasper et al., 2004), analysis of size-frequency distributions (Ramon, M & Richardson, 1992), and analysis of oxygen isotopic composition along the shell growth direction (Keller, N et al., 2002, Gasper et al., 2004), analysis of size-frequency distributions (Ramon, M & Richardson, 1992), and analysis of oxygen isotopic composition along the shell growth direction (Keller, N et al., 2002).

Visible rings and banding patterns are often formed on shells of bivalves when they undergo periods of reduced shell growth (Haag and Commens-Carson, 2008). Ring formation has been associated with conditions that likely affect growth, such as shifts in habitat parameter (e.g., temperature) or intrinsic conditions (e.g., spawning) (Ropes, 1985).



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