Thesis for the Degree of Master of Science

Age determination and growth rate of *Mactra chinensis* (Bivalvia, Mactridae) by external rings and chondrophore growth bands

by

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August 2014

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By
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Abstract

Age, growth and mortality of *Mactra chinensis* were investigated during the period from October 2012 to September 2013 in Busan, South Korea. The relationship between shell length (SL) and height (SH) was related as the following equation, SL= 1.3249SH+0.0162 (r^2=0.9250, n=2463, P<0.001). The relationship between shell length (SL) and total weight (TW) was expressed by the equation, TW=0.0005SL$^{2.6415}$ (r^2=0.8063, n=2472, P<0.001). The monthly variation of the marginal index (MI) of the shell and chondrophore showed that the ring of this species was formed once a year during July. We estimated the age of *M. chinensis* by reading the external rings on the shell and growth bands of chondrophore to compare growth parameters between the two growth characters. The age of this species ranged from 0 to 8 years (shell-based age reading) and from 0 to 10 years (chondrophore-based age reading). Based on external rings and growth bands of

chondrophore for the same period, the von Bertalanffy growth functions were expressed by the equation, L_t = 101.53[1-exp{-0.16(t+0.75)}] and L_t = 90.03[1-exp{-0.20(t+0.50)}], respectively. The likelihood test showed that there was a significant difference in growth parameters between the two methods for L_∞ (P<0.001), K (P<0.001), t_o (P<0.001). Total mortality (Z) and survival rate (S) estimated from chondrophore were 0.692 yr^{-1}, 0.501 yr^{-1}.

1. Introduction

The sunray surf clam *Mactra chinensis* is very fast growing during the first two years of life, and decreases during subsequent years (Fiori 2004). The clam is widely distributed on the upper subtidal sandy bottom off the coast of Korea, Japan, China, Sakhalin, and the maritime province of Siberia (Habe 1977). This clam occurs from the coastal beach zone to depths of over 20 m. It represents one of the most economically important clams in Korea (Ryu and Kim 2001).

There are some studies of the breeding season (Miyazaki 1957 and Tomita 1974), age and growth (Sakurai 1993), morphometry and growth rate (Hanaoka and Shimadzu 1949), effects of water temperature, salinity and substrata on burrowing behaviors (Sakurai et al. 1997). Despite importance of age and growth, little are studies of the age determination and growth characteristic of *M. chinensis*.

Age validation is important in ageing of commercial species (Beamish and MacFarlane 1983, Campana 2001). Panfili et al. (2002) stated that theoretically, a validation should be made of every population of any given species, because there may be important differences between them.

Growth rate and age structure have to provide for fisheries models to assess the health of fishery resource or to correctly interpret 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. Bivalve molluscs have historically been aged by examining the external valve surfaces for dark growth “rings” or “bands” that form as an annual periodic event (Weymouth 1923, Merrill et al. 1966, Feder and Paul 1974).

Ring formation has been associated with factors having probable effect on the metabolism of growth, such as extrinsic environmental conditions (temperature) or intrinsic conditions (spawning) (Ropes 1987). When the aged shells were examined it was very difficult to determine if external lines were annual. The method using the external ring appeared to be fairly accurate at the youngest shells. However, it became more difficult to obtain an accurate age determination at the older and thicker shells (MacDonald and Thomas 1980). Annual microstructural deposits may also occur in other part of the shell that can be prepared for examination by fairly rapid methods, e.g., the chondrophores of the surf clam, *Spisula solidissima*

(Dillwyn) (Ropes and O'Brien 1979).

In this paper we examined the growth banding patterns in shells of the clam *M. chinensis* to determine the age and to estimate growth rate of a population. We also investigated the difference in the growth parameters estimated from shell-based age reading and chondrophore-based age reading.

2. Material and methods

2-1. Sampling

Mactra chinensis used in the study were 2472 individuals collected monthly using a Hydraulic Jet Dredge in Busan, Korea and taken from October 2012 to September 2013. The sampling station was located at a depth of 5m in an area where *M. chinensis* was mainly distributed (Fig. 1). Intact shells from this collection were selected for age and growth analysis from the valve (n=364) and chondrophore (n=372).

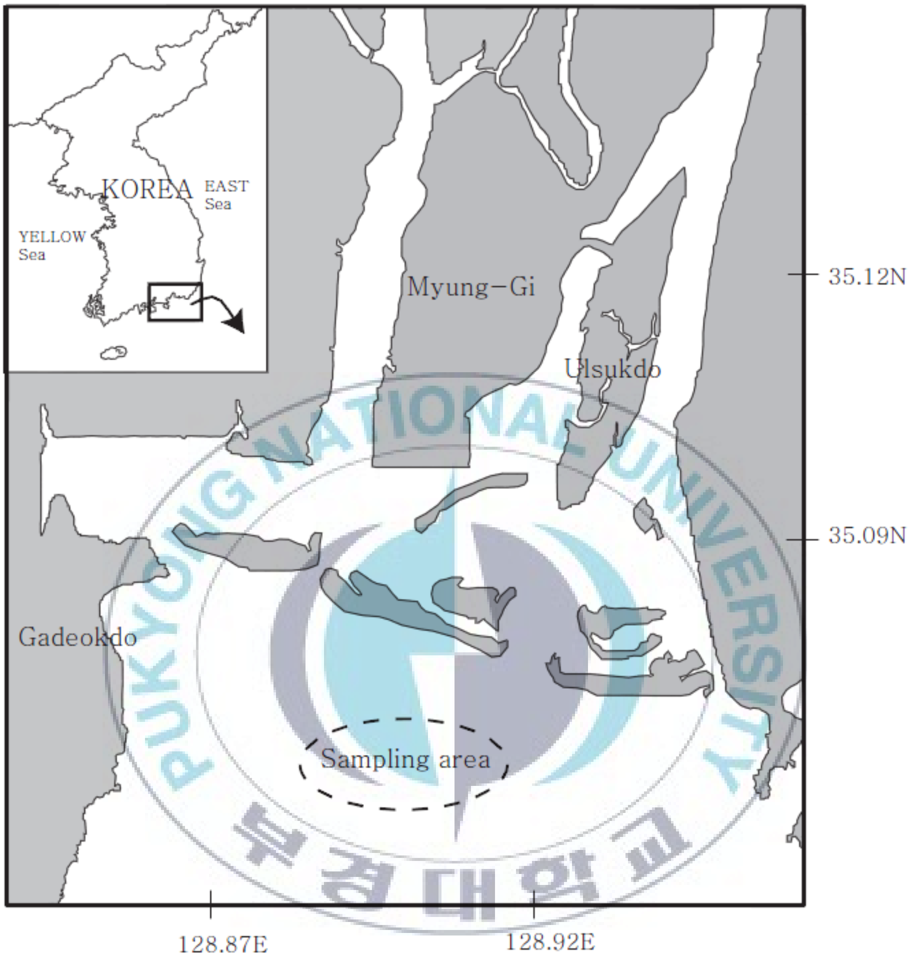

Figure. 1. Map showing sampling site of *Mactra chinensis* in Busan, Korea.

2-2. Morphometric relationship

The shell length (SL) and shell height (SH) of each specimen was measured to the nearest 0.01mm using the Vernier caliper. Conversions among length measurements can generally be accomplished with simple linear regressions models. Length-length relationships were determined by the method of least squares to fit a simple linear regression model.

Total weight (TW) was recorded with an electronic analytical balance to the nearest 0.01g. The relationship between SL and TW was determined by power 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 (*n*= 2472).

2-3. Comparison of calcified structures

The bivalves were opened and all flesh (body) was removed. The shell parts were separated in order to compare age and growth. One of two shells were used for external rings reading and the other for chondrophore growth bands reading.

2-3-1. External ring

The method of peeling off the sheath of the shell with sodium hydroxide (NaOH) is used (Oshima et al. 2004). Because corrosion effect similarity between sodium hydroxide and potassium hydroxide (KOH) was used to increase the appearance of annual growth lines. The shells were exfoliated with a 25% KOH to make the concentric groove on the shell surface easily observable. Growth increments recorded on shell external surface were measured from the umbo to the beginning of the transparent band. The ring number and the shell length at each ring were counted and measured.

2-3-2. Chondrophore growth bands

Age was determined using thin shell sections in which the optic pattern of internal growth bands was crosschecked with the external bands (Fiori and Mors'an 2004).

A pair of shells was selected for sectioning, since it has a single prominent tooth in the hinge. The prepared chondrophore was embedded in the epoxy resin and sectioned along the longest axis across the core at about 0.3mm intervals with a diamond saw. The polishing was tried using #1000-grit grinding paper. Age was determined by counting translucent zones in chondrophore with image processing system consisting of a computer, a video camera microscop (Zeiss DV8), and the Optical Pattern Recognition System software package of Image-Pro Plus Version 4.1.

2-4. Marginal index (MI)

The external surface exhibited an annual growth cycle pattern composed by a succession of translucent and opaque regions (Fiori 2004). Marginal index (MI) analysis was used to validate the annual growth pattern increment deposition (Lai et al. 1996) that is calculated by the equation:

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

where R is the radius of chondrophore, r_n is the length of the chondrophore radius at the time of the nth transparent zone mark in chondrophore.

In external rings method, R is the shell length, r_n is the shell length at the time of the nth transparent zone mark.

2-5. Growth back-calculation and growth curves

Based on the linear regression between chondrophore and shell length, length a growth mark formation were back calculated by the Fraser-Lee equation (Francis 1990) :

$$\mathrm{L} = c + (\mathrm{SL} - c)\left(\frac{\mathrm{S_i}}{\mathrm{S}}\right)$$

where L is the shell length of clam when growth mark i was formed, SL the shell length at time of capture, S_i the distance from chondrophore centre to growth mark i, S chondrophore radius and *c* the intercept parameter.

The following equation was used to relate TL at capture to the chondrophore radius (CR) at capture: TL = *a*+*b*CR, where *a* is the intercept and *b* the slope from the linear regression.

Growth parameters were estimated using von Bertalanffy growth by Walford method and non-linear regression for the *M. chinensis* by the equation:

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

where L_t is the length at age t, L_∞ is the asymptotic length, K the coefficient of growth, t_0 the theoretical age when predicted mean length is zero.

2-6. Mortality rate and survival rate

The survival rate was calculated from the $S=e^{-z}$. Total mortality rate (Z) was estimated from the overall size-frequency distribution and the von Bertalanffy growth function parameters by a size-converted catch curve (Pauly 1984)

$$N_i/\Delta t_i = N_0 e^{-Zt_i}$$

where N_i is the number of individuals in size class i, Δt_i the time required to grow through this size class and t_i the relative age in the mid-size of class i.

2-7. Statistical analysis

The differences in growth curves between the two methods were compared by the method of Kimura’s likelihood ratio test. This method allows the testing of several hypotheses to compare two curves by analyzing one or more growth parameters simultaneously. Cerrato (1990) has shown these methods to be more accurate than traditional approaches in comparing von Bertalanffy parameters.

3. Results

3-1. Morphometric relationship

The samples of *Mactra chinensis* ranged from 27.76 to 80.56mm in shell length (SL) and from 3.6 to 50.66g in total weight. Mean total length and weight (±SD) of shell was 55.64 (±7.32) mm and 22.27 (±8.25) g for each.

The relationship between shell length (SL) and shell height (SH) was SL=1.3249SH+0.0162 (r^2=0.9250, n=2463, P<0.001) and the relationship between shell height (SH) and total weight (TW) was TW=0.0005SH$^{2.6415}$ (r^2=0.8063, n=2472, P<0.001).

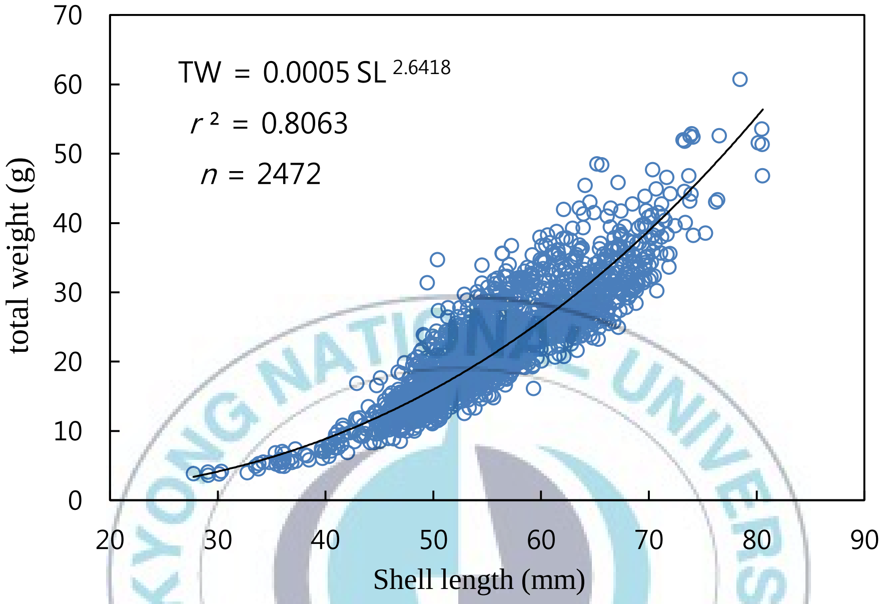

Figure 2. Relationship between shell length (SL) and total weight (TW) of *Mactra chinensis* in Busan, Korea.

3-2. Ring formation

Relationship between shell length and ring radii was observed for the external rings taken from the specimen. The relationship between the chondrophore length and ring radii was examined for the chondrophore growth bands. There were linear relationship between them (Fig. 3, 4).

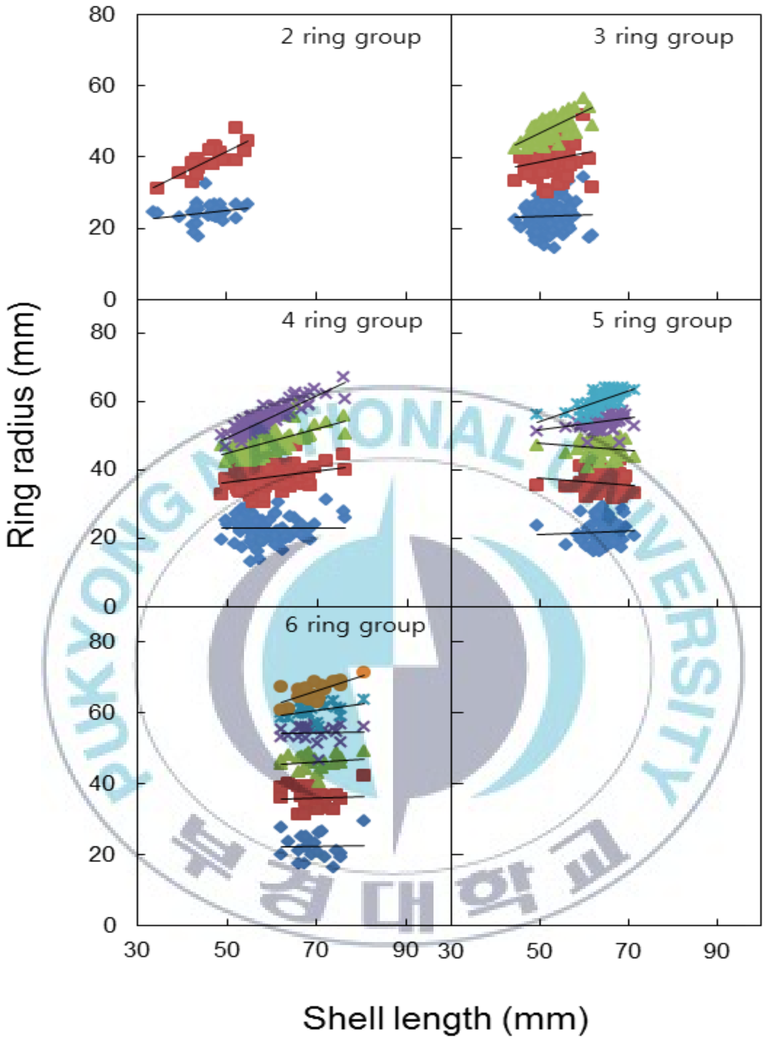

Figure 3. Relationship between shell length and ring radii of *Mactra chinensis* in Busan, Korea.

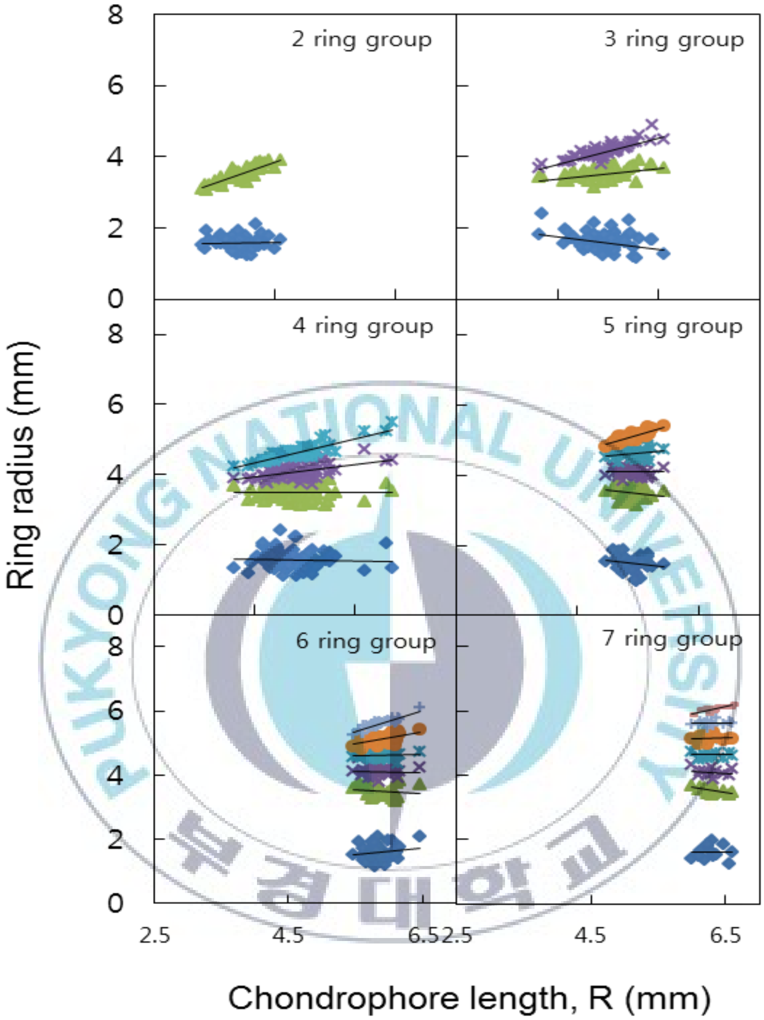

Figure 4. Relationship between chondrophore length and ring radii in the chondrophore of *Mactra chinensis* in Busan, Korea.

3-3. Marginal index (MI)

The mean monthly MI was calculated from specimens for each month. It expressed the relative growth patterns of the shell and chondrophore in a year (Fig. 5). The MI of chondrophore showed the maximum value in October, decreased gradually from November to July and attained the minimum value in July. The MI of shell showed the maximum in September, decreased gradually from October to July and reached the minimum value in July. The MI was the lowest in July.

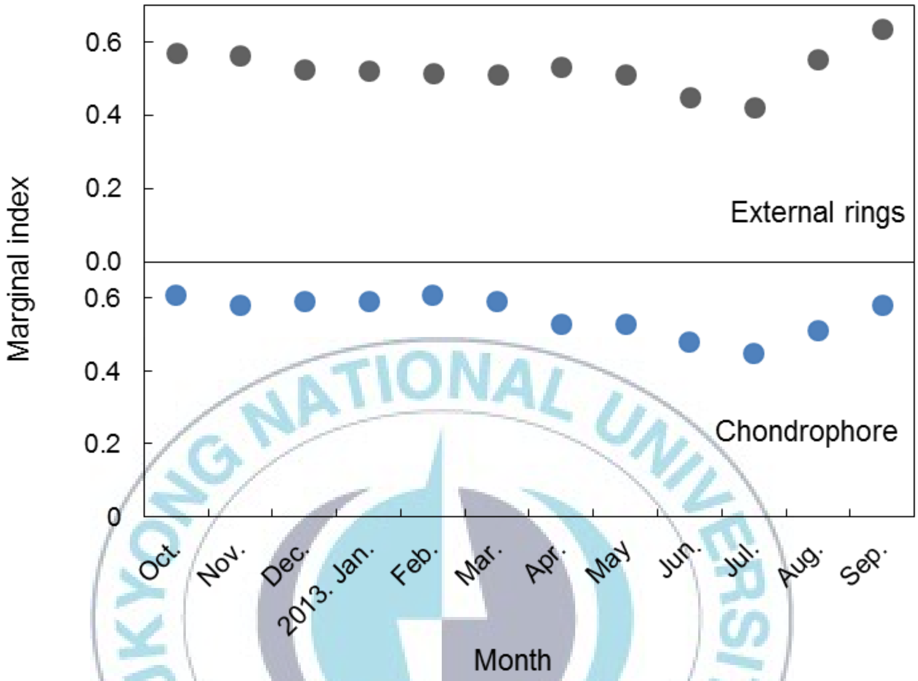

Figure 5. Monthly change of marginal index (MI) of *Mactra chinensis* in Busan, Korea from 2012 to 2013.

3-4. Age composition

The age of *M. chinensis* studied ranged from 0 to 8 years in external ring, and the age group 3 and 4 were the dominant groups. By the chondrophore observation, the age ranged from 0 to 10 years, and the age group 3 and 5 were the prominent groups. Table 1. shows that age reading from chondrophore growth bands was an age older than that from external shell rings.

Table 1. Mean ring radius on the shell and chondrophore of *Mactra chinensis* in Busan, Korea.

	Ring group	Number	Ring diameter							
			r1	r2	r3	r4	r5	r6	r7	r8
Shell length (mm)	1	3	26.01							
	2	24	24.18	39.48						
	3	123	23.38	39.11	47.92					
	4	134	22.96	37.51	47.51	54.24				
	5	58	21.95	36.12	46.68	54.14	57.62			
	6	20	22.20	35.81	45.92	53.08	59.42	66.02		
	7	1	20.98	37.06	46.77	54.38	58.43	66.18	72.36	
	8	1	22.48	35.76	46.42	54.32	57.63	66.13	72.56	76.93
	Mean	364	22.92	37.76	47.18	53.96	58.28	66.33	72.36	76.93

Table 1. Continued.

	Ring group	Number	Ring diameter									
			r1	r2	r3	r4	r5	r6	r7	r8	r9	r10
Chondrophore (mm)	1	9	1.73									
	2	38	1.66	3.52								
	3	80	1.63	3.56	4.13							
	4	113	1.59	3.53	4.15	4.64						
	5	61	1.50	3.51	4.16	4.65	5.12					
	6	38	1.52	3.48	4.08	4.61	5.15	5.59				
	7	17	1.51	3.49	4.11	4.61	5.14	5.61	6.11			
	8	11	1.45	3.47	4.09	4.57	5.09	5.65	6.21	6.58		
	9	3	1.40	3.46	4.01	4.59	5.11	5.59	6.16	6.60	7.14	
	10	2	1.52	3.31	4.15	4.61	5.16	5.59	6.14	6.52	7.21	7.57
	Mean	372	1.55	3.44	4.07	4.58	5.17	5.52	6.18	6.52	7.18	7.57

3-5. Growth back-calculation for total shell length

To estimate growth parameters from the chondrophore, age was determined by a back-calculation method. There was a linear relationship between TL and CR: TL = 5.96CR+14.19 (r^2=0.90, n=372, P<0.001). The total shell height of each clam was back-calculated to the time of formation of the nth annulus from each radius using the equation $\mathrm{L_i} = c + (\mathrm{TL} - c) \times (\mathrm{S_i}/\mathrm{S})$ (Francis 1990) (Table 2).

Table 2. represents the mean shell length at the time of each ring formation in the method using the external shell mark.

Table 2. Back-calculated standard length of *Mactra chinensis* by pooled data in Busan, Korea

	Age	Number	Back-calculated total length									
			r1	r2	r3	r4	r5	r6	r7	r8	r9	r10
Chondrophore (mm)	1	9	24.25									
	2	38	24.02	35.03								
	3	80	23.93	35.22	45.08							
	4	113	23.80	35.08	45.21	54.73						
	5	61	23.51	34.98	45.27	54.81	59.93					
	6	38	23.57	34.85	44.76	54.50	60.18	65.52				
	7	17	23.54	34.89	44.95	54.50	60.09	65.68	70.15			
	8	11	23.35	34.80	44.82	54.18	61.54	63.76	71.75	73.22		
	9	3	23.19	33.17	42.21	52.79	59.85	64.26	70.57	72.31	75.87	
	10	2	23.57	34.06	45.21	54.50	60.26	65.52	70.40	72.73	76.43	79.41
	Mean	372	23.67	34.68	44.69	54.28	60.31	64.95	70.72	72.75	76.15	79.41

3-6. Growth equation

The von Bertalanffy growth equation was estimated from the back-calculation of mean shell height at age of *M. chinensis* (Fig. 6). The estimated VBG parameter L_∞ (asymptotic length) by Walford method was 91.79mm for the chondrophore bands and 93.02mm for the external rings (Table 3).

The estimated VBG parameter L_∞ by non-linear regression was 90.03mm for the chondrophore bands and 101.53mm for the external rings (Table 4).

The r^2 value of external rings and chondrophore by Walford method was represented 0.9905 and 0.9954, respectively. The r^2 value of external rings and chodnrophore by non-linear regression was 0.9928 and 0.9968, respectively.

Table 3. Parameters of the von Bertalanffy growth function estimated by Walford method for shell length (mm) of *Mactra chinensis*, from the age determined by both the analysis of chondrophore growth bands and external shell rings in Busan, Korea.

	L_∞ (mm)	K (y^{-1})	t_0	r^2
External shell rings	93.02	0.20	-0.27	0.9905
Chondrophore growth bands	91.79	0.19	-0.56	0.9954

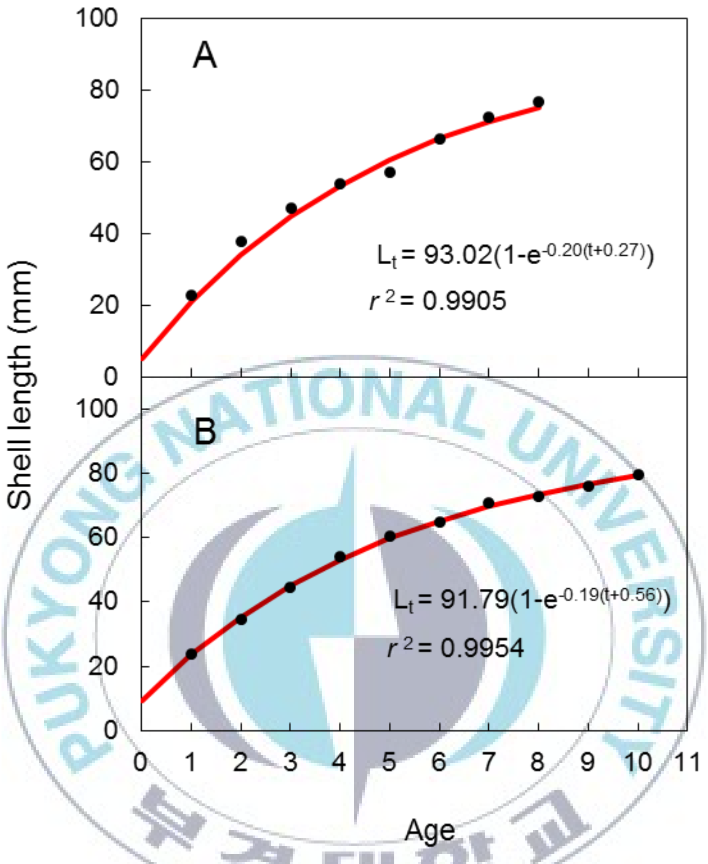

Figure 6. von Bertalanffy growth curves of *Mactra chinensis* estimated by Walford method in Busan, Korea. A, external rings; B, chondrophore growth bands

Table 4. Parameters of the von Bertalanffy growth function estimated by the non-linear regression method for shell length (mm) of *Mactra chinensis*, from the age determined by both the analysis of chondrophore growth bands and external shell rings in Busan, Korea.

	L_∞ (mm)	K (y^{-1})	t_0	r^2
External shell rings	101.53	0.15	-0.75	0.9928
Chondrophore growth bands	90.03	0.20	-0.50	0.9968

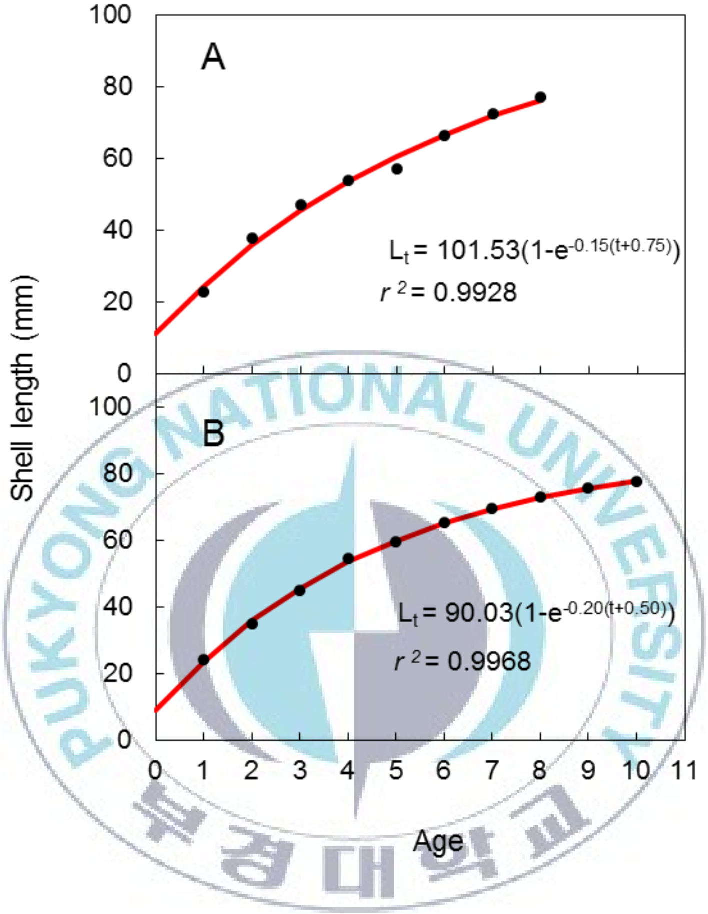

Figure 7. von Bertalanffy growth curves of *Mactra chinensis* estimated by non-linear regression in Busan, Korea. A, external rings; B, chondrophore growth bands.

3-7. Likelihood test

Regression analysis showed that non-linear regression was higher in the r^2 value than Walford method indicating the best fitting of the age data in non-linear regression. So we used non-linear regression to compare the two methods.

The results of likelihood test between the two growth characters indicate that there was a significant difference in all of the three growth parameters L_∞ (P<0.001), K (P<0.001) and t_0 (P<0.001) (Table 5).

Table 5. Likelihood tests comparing von Bertalanffy parameter by non-linear regression estimates for external rings (1) and chondrophore growth bands (2) in Busan, Korea based on data in Table 4.

Hypothesis	Linear constraints	$L_{\infty 1}$	$L_{\infty 2}$	K_1	K_2	$t_{0\,1}$	$t_{0\,2}$	Residual sum of squares (1)	Residual sum of squares (2)	X^2	*P*
H_0	none	90.03	101.53	0.20	0.15	0.50	0.75				
H_1	$L_{\infty1}= L_{\infty2}$	92.58	92.58	0.20	0.19	-0.53	-0.59	25.09	4.73	33.13	<0.001
H_2	$K_1= K_2$	94.44	92.05	0.19	0.19	-0.56	-0.59	24.03	4.43	33.48	<0.001
H_3	$t_{01}= t_{02}$	96.88	91.47	0.18	0.19	-0.59	-0.59	23.30	4.32	33.41	<0.001
H_4	$L_{\infty1}= L_{\infty2}$ $K_1= K_2$ $t_{01}= t_{02}$	91.22	91.22	0.20	0.20	-0.53	-0.53	29.13	6.37	30.93	<0.001

3-8. Mortality and survival rate

For the construction of converted size, only the age-frequency data derived from dredge catches could be used as all the size classes were well represented. Total mortality rate (Z) value of chondrophore growth bands calculated from catch-at-age data was 0.691 $year^{-1}$ (r^2=0.9828) (Fig. 7). Survival rate (S) value estimated from chondrophore was 0.501 $year^{-1}$.

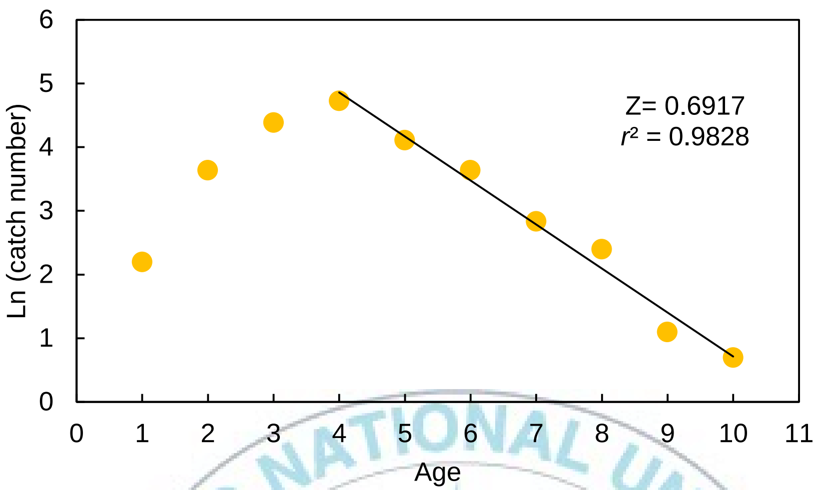

Figure 8. A catch curve with the slope estimating the total mortality rate of *M. chinensis* by chondrophore growth bands in Busan, Korea.

4. Discussion

According to histological and cytological observations of shells gonads, *Mactra chinensis* belongs to summer breeders as most marine bivalves were observed (Chung et al. 1991). This is supported from the results that the spawning peaks of the surf clam, *M. chinensis*, is observed in July. In surf clam the main spawning period occurs from June to July (Chung 1997), the lowest value of MI in this study was consistent with the spawning period.

The spawning period of the Japanese clam *Mactra culcataria* is between May and June in Tokyo Bay (Hanaka and Shimadzu, 1949). Different spawning period of the two surf clam species might be related to water temperature due to the geographical distance (Belding 1993, Chung et al. 1991), time of the food availability (Chung et al. 1991), and some other environmental factors.

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). These factors may also lead to a temporary cessation of growth and cause errors in age determination by examining growth bands (Cardoso 2007).

External rings, internal growth lines and microgrowth bands in bivalve shells have been used to estimate age (Richardson 2001). In many bivalve species surface rings and prismatic shell growth lines are formed annually as a result of seasonal changes in shell deposition (Richardson 2001). We measured the length of chondrophore growth bands and external rings using growth pattern of translucent and opaque zones in order to determine von Bertalanffy growth parameters. The method of aging bivalves according to the alternating patterns of transparency has been used for *spisula sachainensis* in Korea and Japan (Kang and Kim 1983, Sasaki 1981), *Meretrix meretrix* in China (Zhang and Fuxue 1988), and the mussel *Mytilus galloprovinclalis* in Japan (Hosomi 1983).

The asymptotic length (L_{∞}) of *Mactra chinensis* calculated by the von Bertalanffy growth curves was 101.53mm (non-linear regression method) and 71.6mm (Kim 1985) using the shell in the Southern Sea of Korea. On the other hand, it was estimated to 60.02mm in the Yellow Sea (Ryu and Kim 2001). Because the water temperature in the Southern Sea is higher than in the Yellow Sea, the asymptotic length (L_∞) of *Mactra chinensis* of the Southern Sea seems to be higher than that of the Yellow Sea.

The estimated asymptotic length (L_∞) of *spisula solidissima* (Mactridae) was 166mm (in Cardigan Bay) and 141mm (in Northumberland Strait). The annual summer peak of temperature occurred early and the average was 1.3℃ (SD=1.1℃) higher in Cardigan Bay than in Northumberland Strait. The growth of surf clam in Cardigan Bay is exposed to warmer water during the early part of growing season than in the Northumberland Strait (Sephton and Bryan 1990).

External rings method is reliable for determining the age and size of the southern surf clam, caution is required when determining the exact timing of the transitional phases between translucent to opaque zone for specimens collected from death assemblages (Walker and Heffernan 1994). The chondrophore is protected from direct contact with the environmental factors.

In order to compare the results from the two methods the likelihood test was used to detect the difference in the growth rate parameters. Results of likelihood test revealed that there is a significant difference in all of the three growth parameters between two methods L_∞ (P<0.001), K (P<0.001), and t_0 (P<0.001) (Table 4). The residual sum of squares of the growth parameters (L_∞, K, t_0) showed lower values in chondrophore growth bands method than in external rings method.

This study suggests that age determination using chondrophore is more accurate. Estimation of age determination and growth rate using chondrophore was very difficult and takes a long time. Caution is required to prevent damage when sectioning. But when we use chondrophore growth bands method we can obtain more accurate age information. Some bivalves such as *Mya arenaria* (Cerrato et al. 1992), *Spisula solidisma* (Dillwyn) (Ropes and O'Brien 1979) were determined age from the chondrophore. The margin parts of *M. chinensis* are thin and weak, so the distinction of the annual growth lines is hard.

The age information based on observation from external rings method showed that chondrophore was more representative of annual growth bands than the external rings. In case of determining age from external rings, it is available to determine age of surf clams until 7 years old (Belding 1993). Age was not determined for larger clams, because early rings on the valves were obliterated by erosion, and later rings became too crowded together at the valve margin for definite separation (Ropes 1984).

The hinge region in bivalves is the strongest part of the shell, and it is also the only part containing a complete record of growth. These characteristics make the attendance of detailed patterns in the hinge region particularly valuable in archaeological and paleoecological applications (Cerrato 1990). The chondrophores associated with the hinge take place in many bivalve taxa and have been overlooked as potential sources of detailed microgrowth increment records.

We found that *M. chinensis* population from Myung-Gi estuary was estimated life span of 10 years. The Atlantic surf clam ranged in age from 0 to 10 years with two age class dominance in New York (Cerrato and keith 1992). These clams in offshore populations commonly attains ages of 25 and occasionally 30 years (Jones et al. 1978).

We hypothesized that physiological stresses due to reduced salinity and more extreme temperatures fluctuation found under estuarine conditions were probably responsible for the observed differences in population parameters. A substantial body of literature shows that, with decreases in latitude, greater growth rate, earlier age at maturity, and shorter lifespans occur for intraspecific comparisons of marine bivalve species (Newell 1964).

Total mortality estimated from chondrophore in size-frequency at age was 0.6917 (r^2 = 0.9828). The annual probability of survival for surf clams, S, was 0.50 (chondrophore). Because this species was commercially exploited in Busan, mortality due to fishing might be attributed to the reason of high mortality rate. In this study area, the average of dissolved oxygen was low as 5.08 ppm. Because of increasing temperature, phytoplankton multiplication causes decreased dissolved oxygen. It can be threatening clam survival.

Water temperature was the main environmental factor that affected the growth of surf clams in Korea, and there was an evident seasonal change in its growth. It has been reported that the DO, sulphide contents in the bottom and contents of heavy metal ions also had significant effects on the clam (Ma et al. 1997), and especially sharp fluctuations in any environment factor might lead to growth cease and even death of clams (Wu and Lv 1993).

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고마움을 전합니다.

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