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Age and Growth Characteristics of *Okamejei kenojei* in the West Sea of South Korea According to Coronal Vertebral Microstructure

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Abstract: In this study, the growth and age characteristics of *Okamejei kenojei* in the West Sea of South Korea were examined, and specimens were collected by bottom trawls from January to December 2019. The relationship between disc width (L) and body weight (W) was $W = 6.1 \times 10^{-3}\ L^{3.40}$. Age was determined by measuring annuli on the vertebral centrum surface, which forms around June every year. The spawning period was extrapolated from monthly changes of the gonadosomatic index in June–July. The relationship between the vertebral centrum radius and disc width, which was analyzed separately for females and males, was determined as $L = 16.159R^{0.6145}$ and $L = 15.543R^{0.6851}$, respectively. Finally, *O. kenojei* growth, that is, the disc width (L) at a certain age (t), was expressed using the von Bertalanffy growth equation as $L_t = 58.70(1 - e^{-0.21(t+0.12)})$ for females and $L_t = 53.94$ $(1 - e^{-0.26(t+0.05)})$ for males. This study provides basic data on the age characteristics of *O. kenojei* for future research and more efficient fish stock management.

Keywords: *Okamejei kenojei*; ocellate spot skate; age; growth; West Korea Sea; coronal vertebral

Key Contribution: Growth increments in the vertebrae are used to provide accurate age assessments of *Okamejei kenojei* in the West Sea of South Korea.

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1. Introduction

The ocellate spot skate, *Okamejei kenojei* (Rajiformes: Rajidae), is a commercially important fish species that inhabits sandy or tidal flat-bottom habitats (depth: 20–100 m) in the Yellow Sea, East China Sea, East Sea of South Korea, and the Sea of Okhotsk. *O. kenojei* possesses a relatively short snout, wide cartilage that is not easily bent, a round spot at the base of each pectoral fin, with each spot encompassing one to several smaller brown spots, the ventral is white three and there are five rows of dorsal thorns on the tails of males and females, respectively [1].

Information on the age of fish is an important biological parameter for understanding dynamic changes in population growth as it is required to calculate the growth rate, mortality rate, and reproduction [2,3]. The most suitable aging structure may vary from species to species; therefore, the choice of a hard structure for age and growth estimation is vital [4]; age assessment can be divided into four methods: breeding, mark-recapture, estimation of body length composition data, and use of age traits, such as natural growth rings. The age and growth of fish are analyzed using scales, otoliths, vertebrae, and fin rays. Scales and otoliths are used in bony fish; however, if the fish has no scales or the otoliths are too small, vertebrae can be used instead [5], as in a previous study on the age and growth characteristics of *Lophius litulon* (Jordan, 1902) [6]. Since cartilaginous fish have degenerated scales and otoliths are small and easily broken, age cannot be determined using this structure, so age evaluation is performed using vertebrae. Age assessment using vertebrae includes age determination in *Triakis semifasciata* [7], age and sexual maturity in *Platyrhina sinensis* [8], and age and growth estimation in *Leucoraja ocellata* [9]. Age information is important for monitoring and managing fish stock. However, stock management based on inaccurate age assessments can seriously reduce fish populations; thus, it is crucial to increase the accuracy and precision of age assessment in fish. Precise and accurate fish age assessments require calculations of the agreement rate or coefficient of variation between age indicators and various validation approaches [10,11]. In addition, it is important to verify the entire range of ages, including immature individuals [12]. Verification of the first growth ring or annulus, is particularly important because the position of the first annulus represents the starting point for age assessment; thus, incorrect identification of the first annulus may lead to continuing errors in subsequent age readings. Unfortunately, the first annulus is not easily differentiated from structures that are not annuli, which easily leads to errors during age assessment [10]. Despite previous studies on the age and growth characteristics of species in the family Rajidae, including *Raja binoculata*, *Raja rhina* [13], and *Raja clavata* [14] in British Columbia waters, *Beringraja pulchra* [15] in the West Sea, and *Dipturus kwangtungensis* in the northern waters of Taiwan [16], no studies have analyzed the age and growth characteristics of *O. kenojei*. Moreover, current research on the ecology of *O. kenojei* is insufficient. Therefore, the aim of this study is to elucidate the growth and life history of *O. kenojei*. Specifically, we perform age assessments of specimens collected from the West Sea of South Korea using growth increments in the vertebrae. The findings of this study will provide basic data for the rational use and management of *O. kenojei* resources.

2. Materials and Methods

2.1. Fish Sample Collection

Specimens of *O. kenojei* (n = 651) were collected by large pair trawls from January to December 2019 in the West Sea of South Korea (Figure 1). Samples were then transported to the laboratory for subsequent analysis.

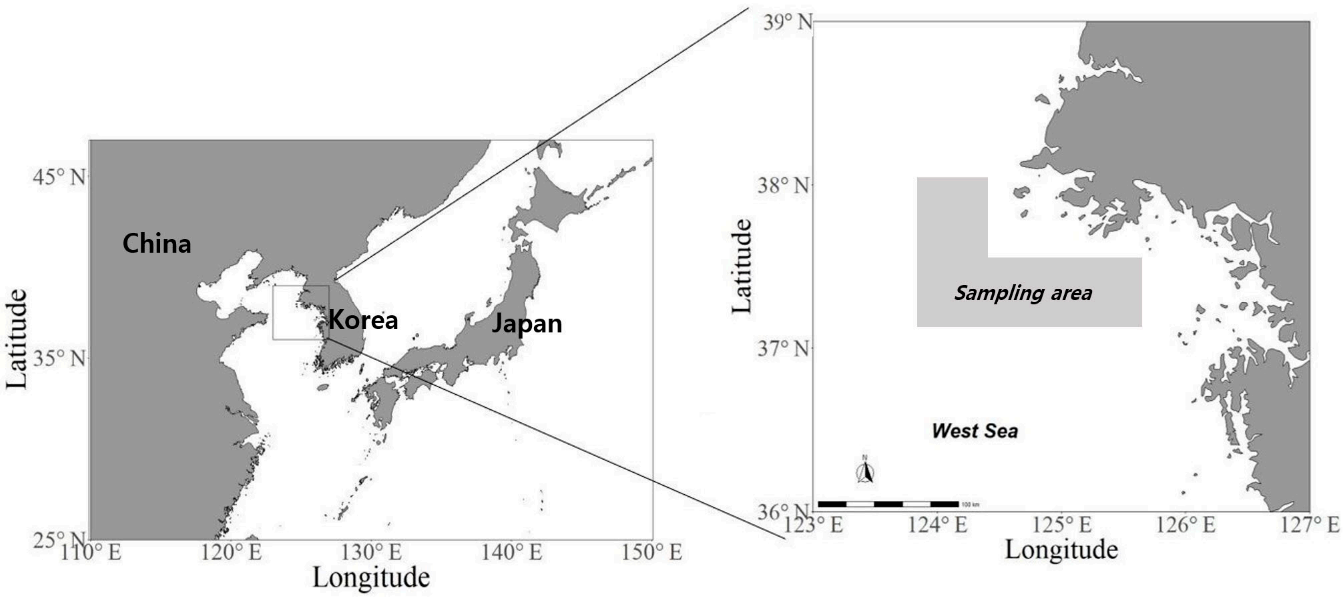

Figure 1. Location of the ocellate spot skate (*Okamejei kenojei*) sampling area in this study.

2.2. Analysis Method

2.2.1. Measurement of Vertebrae Variability

To improve the reliability of the age indicator in this study (annual rings on the vertebrae), we analyzed the variance rate of vertebra size. The diameters of each vertebra were measured in 13 individuals, and the average diameter was obtained for each vertebra. Vertebra 12 exhibited the lowest mean variation in diameter and was used for subsequent age assessment (Figure 2).

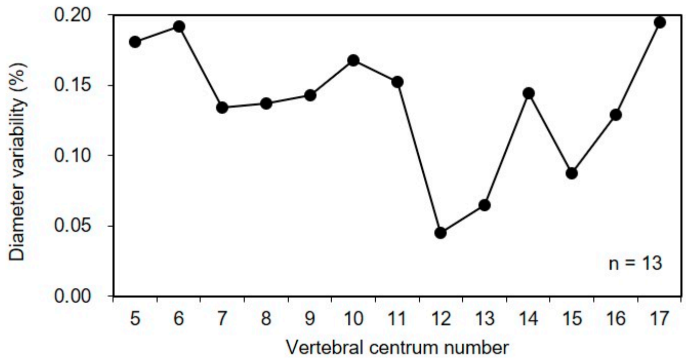

Figure 2. Variability of vertebral centrum diameter for each vertebra.

2.2.2. Vertebrae Preprocessing

The vertebrae were boiled in boiling water for 5 min to remove large impurities, precipitated in a 4% KOH solution to remove the remaining flesh from the vertebrae, then dried. Each vertebral centrum was then stained with a mixture of Alizarin Red S and 70% ethanol at a concentration of 0.1% (w/v) for 5 min and then dried. The dried vertebral centra were placed in a mold after applying a mold release agent (Silicon Mold Release, BUEHLER Co., Lake Bluff, IL, USA). The molding solution, prepared using resin (Castolite Resin, BUEHLER Co.) and hardener (Castolite Hardener, BUEHLER Co.), was accommodated in a molding containing the vertebral centra and dried for 48 h or longer after removing bubbles using tweezers and pins. The molded vertebral centra were then ground to the center of the cross section with a grinder using sandpaper of various roughness values; the cross sections were polished using polishing powder to ensure accurate stain measurement.

2.2.3. Age Assessment and Verification

A stereo microscope (SZX16, Olympus, Tokyo, Japan) and image analysis program (i-Works 2.0, Nahwoo Trading Co., Daejeon, Republic of Korea) were used to measure the vertebral centrum radius (R) and the distance to each growth band (r_n) in mm, and they were measured in three scenarios. A clear boundary was observed between opaque and transparent zones, and the long vertebral centrum radius was confirmed using measurements toward the head of the *O. kenojei*. The first growth band was designated as the first growth ring, and the beginning of each transparent zone was considered to represent a growth band. The vertebral centrum radius (R) was measured as the baseline distance from the focus of the vertebral centrum to the spine bone end. The distance from the focus to each growth band (r_n) was determined along this baseline (Figure 3).

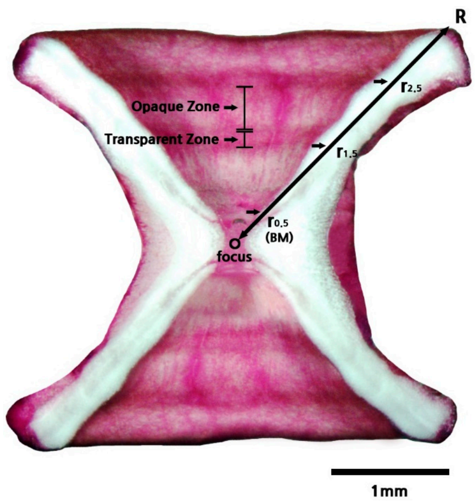

Figure 3. Measurement of the vertebral centrum radius in *Okamejei kenojei*, where r_n represents the distance to the growth ring at = n and r_0 represents the first growth ring.

2.3. Identification of Growth Bands

To examine whether the identified growth bands were appropriate age traits for the age assessment of *O. kenojei*, we verified the agreement between the vertebral centrum radius and the distance from the focus to each growth band, then estimated the band formation time and number of bands according to monthly changes in the marginal index (*MI*):

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

where R denotes the vertebral centrum radius, r_n denotes the distance from the focus to the last growth band, and $r_n{-}1$ denotes the $n - 1$st growth band.

2.4. Spawning Season

To confirm the relationship between the growth band formation period and the spawning period, we used the gonadosomatic index (*GSI*) of *O. kenojei* and egg capsule appearance data [17]. To estimate the spawning period, the disc width (*L*, 0.1 cm), body weight (*BW*, 0.1 g), and gonad weight (*GW*, 0.01 g) of each specimen were measured, and the monthly *GSI* was estimated as follows:

$$GSI = \frac{GW}{BW} \times 100 \quad (2)$$

2.5. Growth Formula

The relationship between the vertebral body radius (*R*) and disc width (*L*) was expressed by the following equation:

$$L = aR^b \quad (3)$$

The von Bertalanffy [18] growth model was used as the growth equation for *O. kenojei* (Equation (4)), along with the inverse average *L*:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \quad (4)$$

Here, *Lt* denotes the disc width at age t, L_∞ denotes the theoretical maximum disc width, K denotes the growth factor, and t_0 denotes the theoretical age when the disc width is zero. A Microsoft Excel 2016 (Microsoft Inc., Washington, DC, USA) solver was used to determine the mature length value, and the constants were estimated by setting the GRG nonlinear least-squares method as the solution, the degree of convergence to 0.0001, the differential coefficient to the center, the population size to 100, and the random initial value to 0.

2.6. Statistical Analyses

To determine the statistical significance of the results, we performed ANOVA tests using the SPSS statistical program (SPSS Inc., Chicago, IL, USA). Differences were considered significant when $p < 0.05$ according to post-hoc Tukey's (*t*) test.

3. Results

3.1. Size and Weight of Specimens

The 651 *O. kenojei* specimens collected from January to December 2019 included 339 females and 312 males (Table 1). The L range was 12.1–35.3 cm for females (mean: 28.0 cm) and 10.5–34.5 cm for males (mean: 28.3 cm). The most frequent maximum L range was 31.0–31.9 cm for both males and females, followed by 28.0–28.9 cm (Figure 4). We observed no significant difference in the mean L values between male and female specimens (*t*-test, $p > 0.05$). The *L*–*BW* relationships showed no significant difference between sexes ($p < 0.05$) and could be estimated as follows: $BW = 3.4 \times 10^{-3}$ L 3.58 ($R^2 = 0.93$; $N = 339$; $p < 0.05$) for females; $BW = 5.7 \times 10^{-3}$ *L* 3.66 ($R^2 = 0.89$; $N = 312$; $p < 0.05$) for males; and $BW = 6.1 \times 10^{-3}$ L 3.40 ($R^2 = 0.93$; $N = 651$; $p < 0.05$) for both sexes (Figure 5).

Table 1. Number and size (disc width) of ocellate spot skate (*Okamejei kenojei*) individuals collected from the West Sea of South Korea throughout 2019.

Year	Month	No. of Fish (*N*) Female	Male	Disc Width Range (Mean (cm) ± SD) Female	Male
2019	Jan.	30	30	22.1–33.5 (27.5 ± 2.9)	23.5–32.1 (28.0 ± 2.4)
	Feb.	30	30	28.9–35.3 (31.7 ± 1.8)	27.3–34.5 (30.6 ± 1.7)
	Mar.	30	30	17.8–31.8 (23.9 ± 3.3)	24.4–34.3 (29.7 ± 2.2)
	Apr.	30	29	22.7–33.7 (28.7 ± 3.1)	21.7–32.4 (28.5 ± 2.9)
	May	32	33	12.1–33.2 (28.6 ± 5.8)	10.5–33.3 (28.9 ± 6.3)
	Jun.	30	23	21.0–32.0 (27.3 ± 2.2)	19.3–32.9 (27.5 ± 3.4)
	Jul.	30	29	26.7–33.5 (30.6 ± 1.8)	25.5–31.3 (28.8 ± 1.5)
	Aug.	7	13	24.5–31.6 (27.8 ± 2.3)	22.3–31.0 (27.2 ± 2.2)
	Sep.	30	15	21.0–33.6 (26.6 ± 2.8)	20.2–29.1 (25.4 ± 2.6)
	Oct.	30	20	22.9–33.1 (28.6 ± 2.2)	23.1–31.3 (27.8 ± 2.1)
	Nov.	30	30	20.7–35.0 (26.9 ± 4.5)	21.0–30.5 (26.2 ± 2.2)
	Dec.	30	30	23.6–32.5 (27.9 ± 2.1)	26.1–33.4 (28.8 ± 1.9)
Total		339	312	12.1–35.3(28.0 ± 3.7)	10.5–34.5(28.3 ± 3.3)

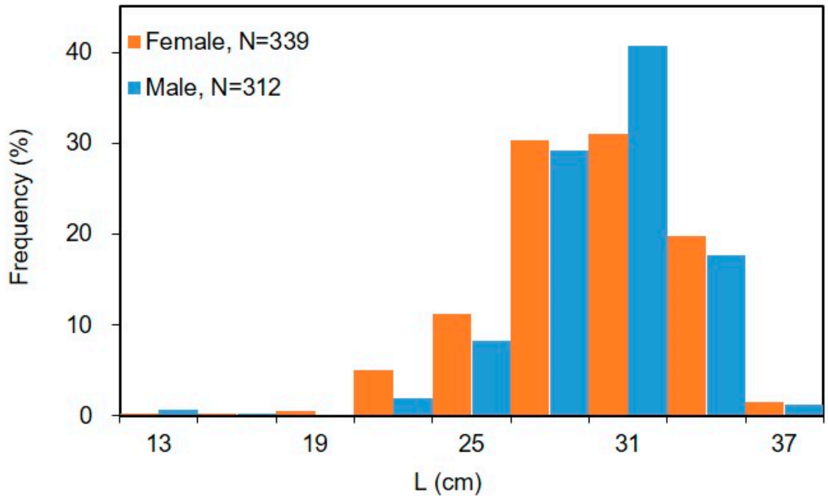

Figure 4. Frequency distribution of disc width measurements of *Okamejei kenojei* collected from the West Sea of South Korea.

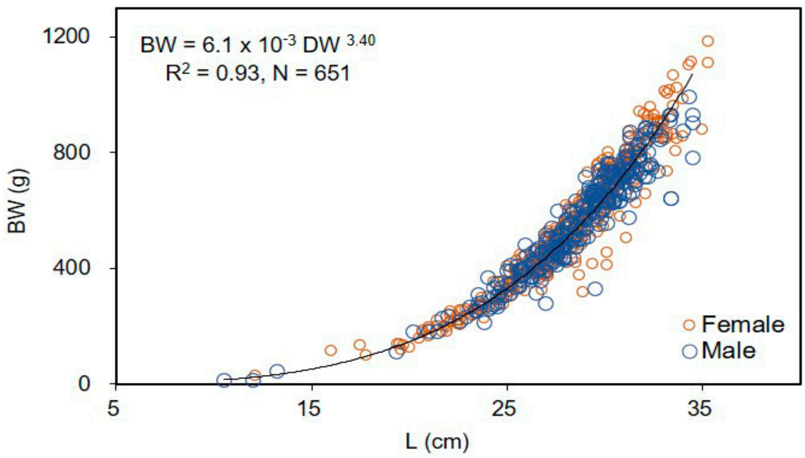

Figure 5. Relationship between body weight (*BW*) and disc width (*L*) of *Okamejei kenojei* collected from the West Sea of South Korea.

3.2. Growth Bands

Growth band measurements were derived from a total of 651 vertebral centra (339 females and 312 males). To confirm whether the growth bands on the vertebral centra were suitable for use as annuli and to investigate the accuracy of growth band measurements, we determined the relationship between the vertebral centrum radius and the distance to the growth band (Figure 6). Specimens were clearly distinguished according to the number of rings, and each growth band was separated by rectilinear regression with respect to the vertebral centrum radius.

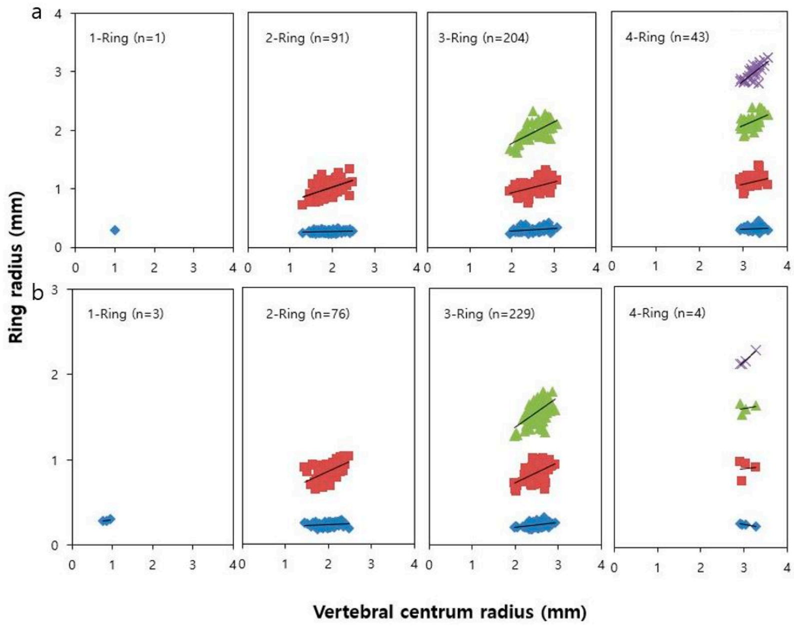

Figure 6. Relationship between vertebral centrum radius and growth ring radius for (**a**) female and (**b**) male specimens of *Okamejei kenojei* collected from the West Sea of South Korea.

3.3. Monthly Change in Marginal Index

Monthly changes in the marginal index were investigated to confirm the time and number of growth band formations throughout the year. The marginal index of females reached a maximum in April and then gradually decreased, reaching a minimum in June. The marginal index of males started to increase in February and showed the lowest value in June (Figure 7). The Marginal index of males and females showed a minimum value around June, which coincided with the transition between opaque and transparent zones in the images; the growth band formation occurred once a year. Therefore, the growth bands were identified as annual rings.

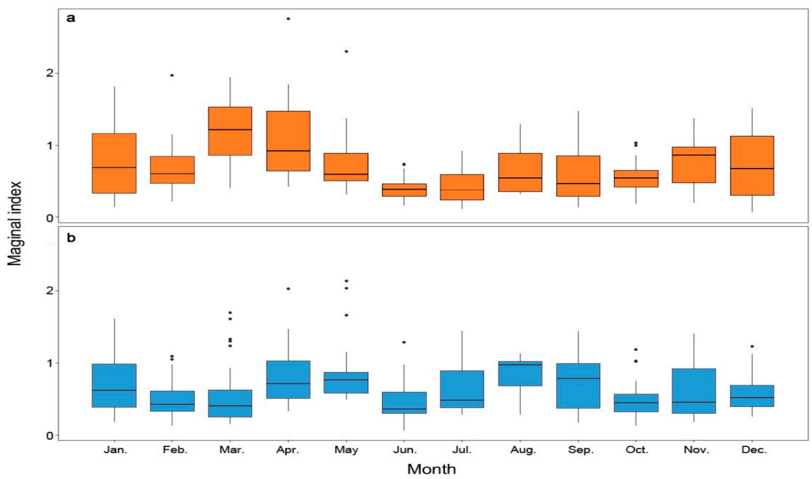

Figure 7. Monthly variation of the marginal index (mean and standard deviation) of the vertebral centra in (**a**) female and (**b**) male specimens of *Okamejei kenojei* collected from the West Sea of South Korea.

3.4. Monthly Change in Gonadosomatic Index (GSI) and Female Egg Capsule Appearance

Monthly changes in the female *GSI* and marginal index were analyzed to determine the relationship between the time of the first annulus formation and the spawning period. The *GSI* started to increase in March, reaching a maximum in May, then gradually started to decrease from May, reaching a minimum in August. Therefore, the spawning season for *O. kenojei* was estimated as May–August, with the main spawning season in July (Figure 8).

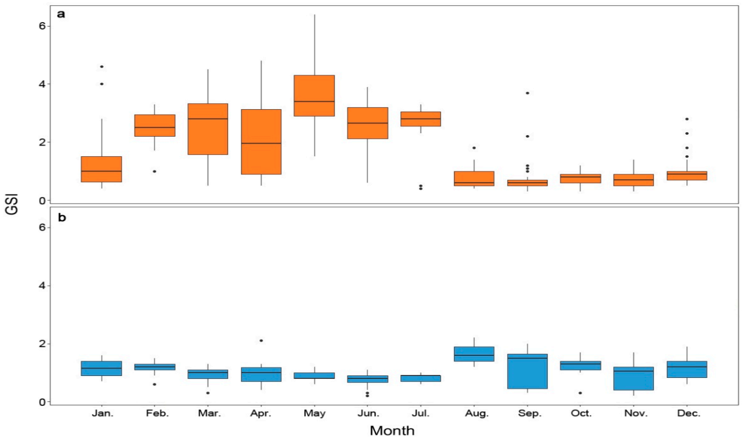

Figure 8. Monthly variation in the gonadosomatic index (*GSI*) of (**a**) female and (**b**) male specimens of *Okamejei kenojei* collected from January to December 2019 from the West Sea of South Korea.

Analysis of the gonads of female *O. kenojei* revealed that the egg capsule, which can be used as an indicator of the spawning season, did not appear in January–February or October–December but mainly in March–September. Therefore, the main spawning season for *O. kenojei*, according to egg capsule appearance, was estimated to be May–July (Table 2).

Table 2. Egg capsule appearance rate in female *Okamejei kenojei* collected from the West Sea of South Korea.

Year	Month	Number of Individuals (A)	Number of Individuals Possessing Egg Capsules (B)	Egg Capsule Appearance Rate (B/A, %)
2019	Jan.	30	-	0.0
	Feb.	30	-	0.0
	Mar.	32	1	3.1
	Apr.	30	3	10.0
	May	43	9	20.9
	Jun.	30	15	50.0
	Jul.	30	21	70.0
	Aug.	7	-	0.0
	Sep.	30	1	3.3
	Oct.	29	-	0.0
	Nov.	30	-	0.0
	Dec.	30	-	0.0

3.5. Back Calculation of Disc Width and Body Weight When Forming a Growth Band

Based on the *GSI* and egg capsule appearance time of *O. kenojei*, we estimated that the main spawning season was July, and the annulus formation period was June. Due to it taking an average of five months from spawning to hatching [19], the first annulus formation period was estimated to be June of the following year after hatching. As a result, the estimated age of *O. kenojei* at the time of the formation of the first annulus was 0.5 years.

To inversely calculate the disc width (L) at the time of crest formation, we determined the relationship between the vertebral centrum radius (R) and L (Figure 9). Using the equations below, we backcalculated the average disc width at the time of formation of female and male growth bands:

$$Female : L = 16.159R^{0.6145}\left(r^2 = 0.8252\right) \quad (5)$$

$$Male : L = 15.543R^{0.6851} \left(r^2 = 0.8039\right) \quad (6)$$

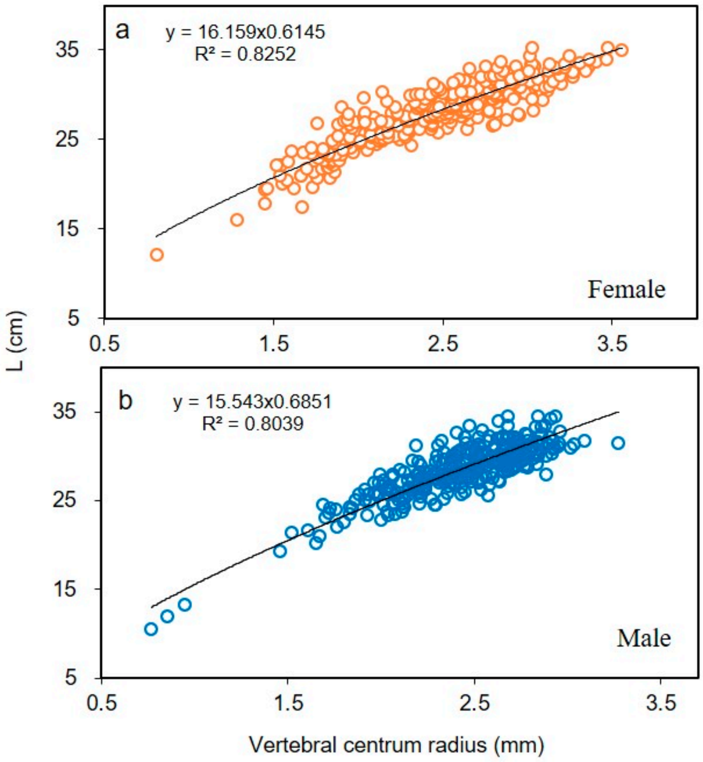

Figure 9. Relationship between vertebral centrum radius and disc width of (**a**) female and (**b**) male specimens of *Okamejei kenojei* collected from the West Sea of South Korea.

3.6. Growth Equation Estimation

As shown in Table 3, the sum of the squared errors of the growth equation, which represents the error range of the data, was 0.307 for females and 0.108 for males. The theoretical maximum disc width estimated by the von Bertalanffy [18] growth model was 38.88 cm for females and 38.54 cm for males at five years. However, the maximum estimated lengths used in this study were 35.30 cm for females and 34.50 cm for males, which are 3.58 cm and 4.04 cm larger, respectively. The estimated growth coefficient was 0.21 per year for females and 0.26 per year for males, indicating less growth for females than for males, and *t*0 was −0.12 for females and −0.05 for males (Table 3).

Table 3. von Bertalanffy growth equation (VBGE) results for *Okamejei kenojei* collected from the West Sea of South Korea (SS: sum of squares of deviation; L_∞: theoretical maximum disc width; K: growth coefficient; SSQ: sum of squared error).

t (year)	Back-Cal. Disc Width (cm)	VBGE Disc Width (cm)	Residual	SS	L_∞ (cm)	K	t_0	SSQ
							Female	
0.5	7.32	7.22	0.099	0.010	38.8	0.21	−0.12	0.307
1.5	16.72	17.06	−0.343	0.118				
2.5	25.41	25.02	0.395	0.156				
3.5	31.30	31.45	−0.151	0.023				
							Male	
0.5	7.10	7.04	0.056	0.003	39.08	0.26	−0.05	0.108
1.5	17.41	17.61	−0.200	0.040				
2.5	26.04	25.80	0.238	0.056				
3.5	32.05	32.14	−0.093	0.009				

Therefore, the growth equations for males and females for the theoretical maximum disc width estimated using nonlinear regression parameters were as follows:

$$Female : L_t = 58.70\left(1 - e^{-0.21(t+0.12)}\right) \quad (7)$$

$$Male : L_t = 53.94\left(1 - e^{-0.26(t+0.05)}\right) \quad (8)$$

Thus, the disc widths by age estimated by the von Bertalanffy [18] growth formula were as follows: $L_{0.5}$ = 7.22 cm, $L_{1.5}$ = 17.06 cm, $L_{2.5}$ = 25.02 cm, and $L_{3.5}$ = 31.45 cm for females and $L_{0.5}$ = 7.10 cm, $L_{1.5}$ = 17.70 cm, $L_{2.5}$ = 25.91 cm, and $L_{3.5}$ = 32.06 cm for males (Figure 10).

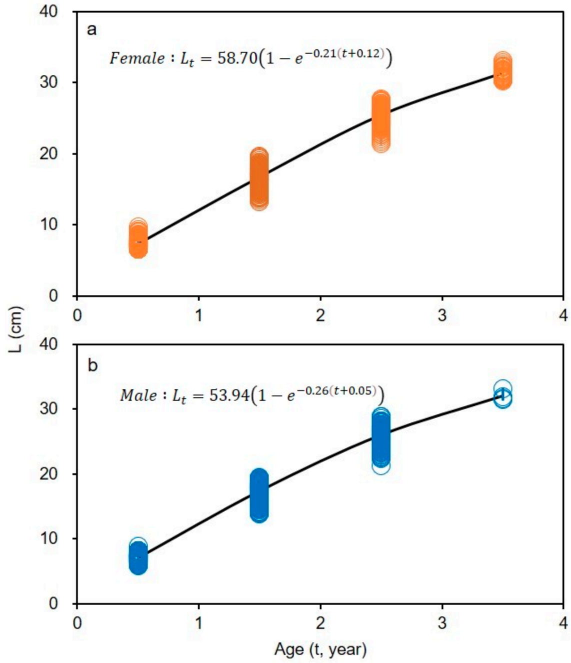

Figure 10. Theoretical von Bertalanffy growth curves of disc width for (**a**) female and (**b**) male specimens of *Okamejei kenojei* collected from the West Sea of South Korea. Vertical bars represent standard deviations.

4. Discussion

Basic data of fish age, composition, and structure are essential for efficient stock management in fisheries; therefore, we present a method for identifying *O. kenojei* age according to vertebrae annual rings. We found that the annual ring in *O. kenojei* is formed at the start of June every year. In this study, *O. kenojei* formation started in June every year as the *MI* value that was referred to along with the water temperature. Based on the *O. kenojei* samples collected in 2019, the average water temperature for the past four years was obtained using NIFS data. The average water temperature in June, when growth bands were formed, was confirmed as 9.7 °C, suggesting that the formation of *O. kenojei* growth bands may be affected by the water temperature. The reason for low catches of *O. kenojei* in periods of high water temperatures is that activity is low, and feeding is reduced. Therefore, the opaque zones form during periods of slow growth. This behavioral trend has previously been observed in *Beringraja pulchra*, which is a cold-water fish species [20].

In general, the causes of band formation in fish include spawning, injury, or an irregular diet [21]. However, by comparing the *GSI*, egg capsule appearance, and time of growth band formation in *O. kenojei*, we confirmed that the timing of growth band formation and the main spawning season were similar. Therefore, the period between a fish spawning from the mother and developing the first annulus is one year. Moreover, the results indicate that a water temperature increase leads to growth band formation, which indirectly affects the fish spawning season. Similarly, the spawning period and growth band formation period were also similar in previous studies on *Lophius litulon* [6] off the coast of Korea, *Kareius bicoloratus* [22] in the West Sea of South Korea, and *Mugil cephalus* [23] off the coast of Yeosu, South Korea. The growth and spawning seasons of *O. kenojei* are also considered to be indirectly affected by water temperature.

Currently, many studies are being conducted on the age and growth characteristics of Rajidae fish species; however, no studies have used *O. kenojei* as the target species. According to a comparison of *O. kenojei* with other domestic and foreign Rajidae species (Table 4), the maximum age of *O. kenojei* is 3.5 years, whereas that of *Raja pulchra* living in South Korea is 4.25 years, with a theoretical maximum disc width (L_∞) for males and females of 101.73 cm and 72.49 cm, respectively [15]. The maximum age of *Dipturus chinensis*, which lives in the East China Sea, including off of Japan, is 15 years for females and 13 years for males, with L_∞ values of 83.1 cm for females and 76.8 cm for males [24]. Unlike these studies, the age assessment of *Raja clavata* in the North Atlantic was conducted using caudal spines rather than vertebrae. The maximum age and L_∞ values for *Raja clavata* are 10 years and 140.7 cm and 117.1 cm for females and males, respectively [14] (Table 4). In this study, male and female growth rates were estimated to be similar in comparison to those of other fish species. In this study, the growth rates of males and females were comparable to those of other fish species, which may be due to the insufficient number of samples based on skate ages. As of 3.5 years, 43 females and 4 males were sampled, and the failure to sample sufficiently large individuals may have led to the above growth rate, requiring additional research with samples of skates by size. Because of the narrow length range of specimens included in this study, future research should sample a wider range of individuals. Regarding other *Rajidae species*, *Beringraja pulchra*, *Dipturus chinensis*, and *Raja clavata* are mostly large fish species, with a maximum age exceeding 10 years (except for *Raja pulchra)*. Therefore, unlike in other countries, *O. kenojei* and *Raja pulchra* are caught as commercial fish species in South Korea. It may be likely that the maximum age cannot be estimated because of factors affecting fish growth, such as overfishing, the water temperature of habitats in each sea area, prey organisms, and low-quality environments.

Table 4. Comparison of growth parameters and growth performance indexes among different studies estimated by the von Bertalanffy growth equation.

Species	Locality of Study		L_∞ (cm)		K	t_0	Max. Age (years)	Material	Reference
Okamejei kenojei	West Sea, South Korea	F M	38.8 39.1	(L) (L)	0.21 0.29	−0.12 −0.05	3.5	Veterbral	This study
Beringraja pulchra	West Sea, South Korea	F M	101.7 72.5	(L) (L)	0.35 0.55	0.09 0.08	4.25	Veterbral	[15]
Dipturus chinensis	Northern East China sea	F M	83.1 76.8	(TL) (TL)	0.103 0.109	−1.20 −1.28	15 13	Veterbral	[24]
Raja clavata	Northern Atlantic Current	F M	140.7 117.1	(TL) (TL)	0.097 0.142	−0.88 −0.36	10	Caudal thorn	[14]

The results of this investigation into the age and growth characteristics of *O. kenojei* have important implications for resource evaluation, resource management, and future research. Moreover, our findings can be used as basic data for future studies on the ecology of *O. kenojei* and the factors influencing growth.

5. Conclusions

This study was to perform accurate age assessments of *O. kenojei* using growth increments in the vertebrae. Age was determined by blind slide mid-vertebral surface reading, and an annular ring was formed once a year around June. The spawning season was estimated based on the monthly gonadal constitutional index changes from June to July. The relationship between the central radius of the spine and the intervertebral disc width was analyzed separately according to gender, with $L = 16.159\ R^{0.6145}$ for women and

$L = 15.543\ R^{0.6851}$ for men. The research results showed that *O. kenojei* was 3.5 years old, and the vertebrae could well reflect the age and growth of *O. kenojei*.

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Institutional Review Board Statement: The study was conducted in accordance with the regulations on research ethics of the National Institute of Fisheries Science. We only used specimens from the fish market which were already dead.

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