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Age and Growth of Turbot *Psetta maxima* in the Black Sea, Turkey

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Abstract

The age and growth of turbot *Psetta maxima* in the Black Sea were examined from specimens caught by both coastal fisheries in Trabzon and a trawl net survey of the Trabzon Fisheries Research Institute from December 1997 to July 1999. The number of otoliths available for examination was 641 pairs of otoliths. We counted the number of otolith opaque and hyaline zones and measured the otolith and ring radii. Monthly changes in the ratio of the number of opaque edges and marginal growth rate indicated that a single ring was formed once a year for both sexes. The relations of back-calculated standard length(cm) $L(t)$ to age t(years old) expressed using the Bertalanffy growth equation were represented as $L(t)=54.8\{1\text{-}e^{-0.481(t+0.011)}\}$ and $L(t)=45.0\{1\text{-}e^{-0.597(t+0.011)}\}$ for female and male, respectively. The estimate of growth coefficient did not differ from those of turbot in the Gulf of Lion and in a release-recapture experiment in the Black Sea, but were nearly twice as large as that in the Gulf of Pomerania.

Key Words: turbot, *Psetta maxima*, Black Sea, age determination, growth

Introduction

Turbot *Psetta maxima* is a major target species of the gill net fishery in the Black Sea coastal waters off Turkey. The species is widely distributed in European coastal waters in the northern Atlantic from Norway to Morocco, including the Baltic, Mediterranean and Black Seas (Blanquer et al., 1992). A decrease in turbot catch in the coastal waters off Trabzon prompted Trabzon Central Fisheries Research Institute (TCFRI) and Japan International Cooperation Agency (JICA) to undertake "The Fish Culture Development Project in the Black Sea" from 1997. The information on the growth and age of fish is indispensable for the management of the target population. However, there are few studies to date on the growth of turbot in the Black Sea. Szlakowski (1990) and Robert and Vianet (1988) investigated the relationship between the body length and age of turbot in the Gulf of Pomerania, Baltic Sea, and in the Gulf of Lion, Mediterranean Sea, respectively.

This paper focuses on age determination and the growth of turbot caught in the coastal area of the Black Sea off Trabzon.

Materials

The specimens used in this study were caught both by gill nets of the coastal fishermen and the trawl net survey of TCFRI from December 1997 to July 1999, of which the total number was 1056. Individuals were classified according to sex, and we measured total length (mm), standard length (mm), body weight (g) and gonad weight (g). A pair of otoliths was removed from each individual, but only 641 pairs were available for examination (female, 330; male, 311).

The ocular side otolith (Sagitta) was used in this study to observe the otolith rings, although the rings which was on some relative thick otoliths were not discernible and thus unable to be measured. Accordingly, the thick portion of these otoliths was polished by a metal file until thin enough for discernment of the rings. All otoliths were washed and stored in distilled water. The opaque zones of each otolith were observed and counted under a binocular microscope using incident light.

In addition, a tag-recapture experiment was carried out by TCFRI in 1998. The mean total length was 18.3 cm at release on March 8 1998. Seven turbot were recaptured in the period of April 2000 to June 2001 (Table 1), and their total lengths were measured.

Table 1. Date and total length at capture of seven turbot released on March 8 1999.

Specimen Number	Capture date	Total length at capture(cm)
1	Jan. 11, 2001	51.1
2	Jan. 9, 2001	45.9
3	Jun. 16, 2000	37.5
4	May. 25, 2000	38.1
5	May. 11, 2000	39.6
6	May. 11, 2000	32.5
7	Apr. 15, 2000	35.7

Methods

First, we defined the otolith radius R (mm) as shown in Figure 1, where R is the maximum straight line distance from nucleus to the outer edge of the otolith anterior projection. The straight line was used as the course line for measuring the radius of the ith ring, r_i (mm). Ring radius r_i was defined as the distance from the nucleus to each outer edge of the ith opaque zone on the course line (Figure 1). The otolith and ring radii were measured to the nearest 0.1 mm.

Second, the formative period of opaque zone and its number formed with a single year were determined so as to clarify the availability of otolith for age determination of turbot. In addition, the marginal growth rate I of otolith was used to determine the formative period of the opaque zone, which was calculated as follows;

$$I = \frac{R - r_i}{r_i - r_{i-1}} . \qquad (1)$$

Third, we estimated the relationship between the otolith radius and ring radius. Using this relationship and relation of the otolith radius to the observed standard length, we estimated the back-calculated standard length when the ring was formed.

Fourth, using the above results, we estimated the relation of the standard length L (t) to age t. Here, we adopted the Bertalanffy growth equation (Bertalanffy, 1938),

$$L(t) = L_\infty \{1 - e^{-k(t-t_0)}\}, \qquad (2)$$

where L_∞, k and t_0 are the asymptotic standard length (mm), growth coefficient (year^{-1}) and age at which the back-calculated standard length is 0, respectively. Since, turbot prelarvae are 0.3 cm in standard length, we assumed that L (0) was equal to 0.3 cm. The non-linear least squares method with a constraint was applied for the estimation of the three parameters. That is, we estimated L_∞, k and t_0 to minimize the weighted sum of least squares SS (L_∞, k, t_0),

$$SS(L_\infty, k, t_0) = \sum_{t=0}^{T} n_t [L(t) - L_\infty \{1 - e^{-k(t-t_0)}\}]^2 , \qquad (3)$$

subject to

$$L(0) = L_\infty (1 - e^{kt_0}) , \qquad (4)$$

where n_t and T are the number of specimens at t years old and the maximum age of each sex, respectively.

Finally, we compared the estimate of k obtained above with that calculated from the tag-recapture data of turbot in the Black Sea. The total length in the tag-recapture data was converted to the standard length on the assumption that the relation of standard length to total length was the same as that in the specimens collected by both the commercial and TCFRI.

Let us denote the release and recapture dates of the ith individual by $t_{r,i}$ and $t_{c,i}$, respectively. Here, $t_{r,i}$ is constant, because all fish were released in the same day. From Eq. (2), the increment of length ΔL_i can be rewritten as

$$\Delta L_i = \{L_\infty - L(t_{r,i})\}(1 - e^{-k\Delta t_i}) , \qquad (5)$$

where Δt_i is $t_{c,i}$ - $t_{r,i}$ (Quinn and Deriso, 1999). We estimated k in Eq. (5) using a non-linear regression method.

Results

Otolith characteristics

The anterior surface of the ocular side otolith had a characteristic projection (Figure 1). On the other hand, the blind side otolith was more circular without any obvious protrusions. The anterior protrusion on the ocular side otolith appeared to grow relatively larger with age, and the interval between rings became wider than on the blind side otolith. Accordingly, we employed the ocular side otolith for age determination.

The otolith had hyaline and opaque zones lying concentrically around a nucleus. However, almost all otoliths had alternating thick and thin opaque zones (Figure 1). Thus, we counted and measured the number of opaque zones and the distance from the nucleus to the outer edge of each opaque zone.

Formation of opaque zone

Figure 2 represents the ratio of the number of otoliths which had a thick opaque zone on the outer margin to the total number of otoliths. The opaque edge began to appear from October and the above ratio increased with month to April or May in female. The ratio reached maximum level in these months, and decreased rapidly to September. The corresponding ratio in male started to increase from September, reached at maximum level in May and thereafter decreased. These facts indicate that the thick opaque zone and hyaline zone were formed from February to May and from June to September for both sexes, respectively. That is, the thick opaque zone indicates an annual ring.

The closed circles in Figure 3 represent the marginal growth rate of female and male if the thick opaque zone is only regarded as the otolith ring. The rate in female decreased from January to May, but thereafter increased. The same tendency was observed for male, that is, the marginal growth rate in May was the lowest recorded. The rate increased after that month. The above facts suggest that the thick opaque zones of female and male were formated once in a year, in the period between March and June, when the

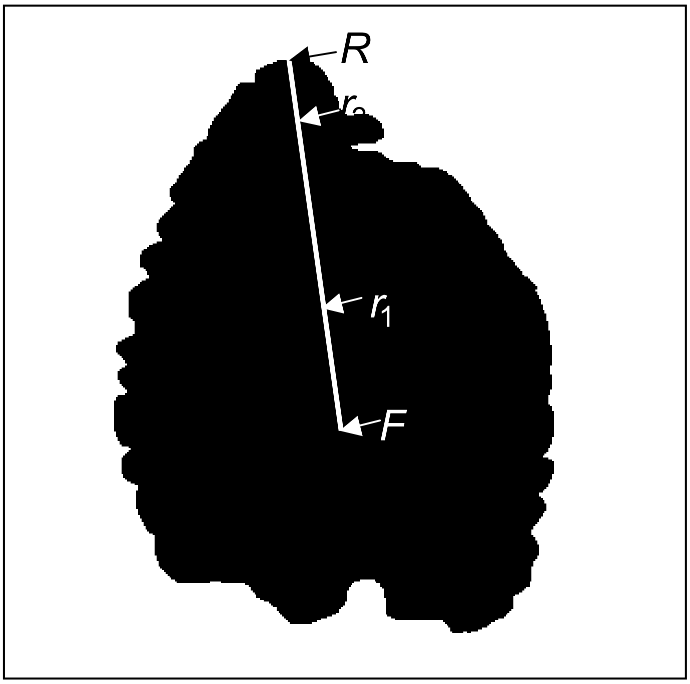

Figure 1. Ocular side otolith of turbot. *F*, *R* and r_i(i = 1, 2, ···) indicate the focus, the otolith radius and the ring radius, respectively.

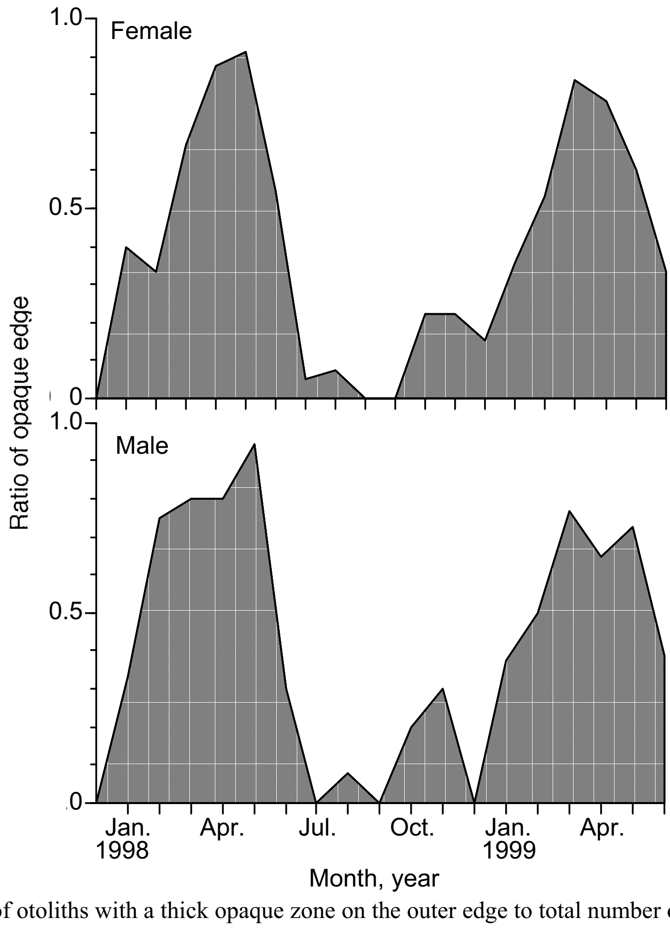

Figure 2. Ratio of number of otoliths with a thick opaque zone on the outer edge to total number of otoliths for turbot in the Black Sea. Top, female; bottom, male.

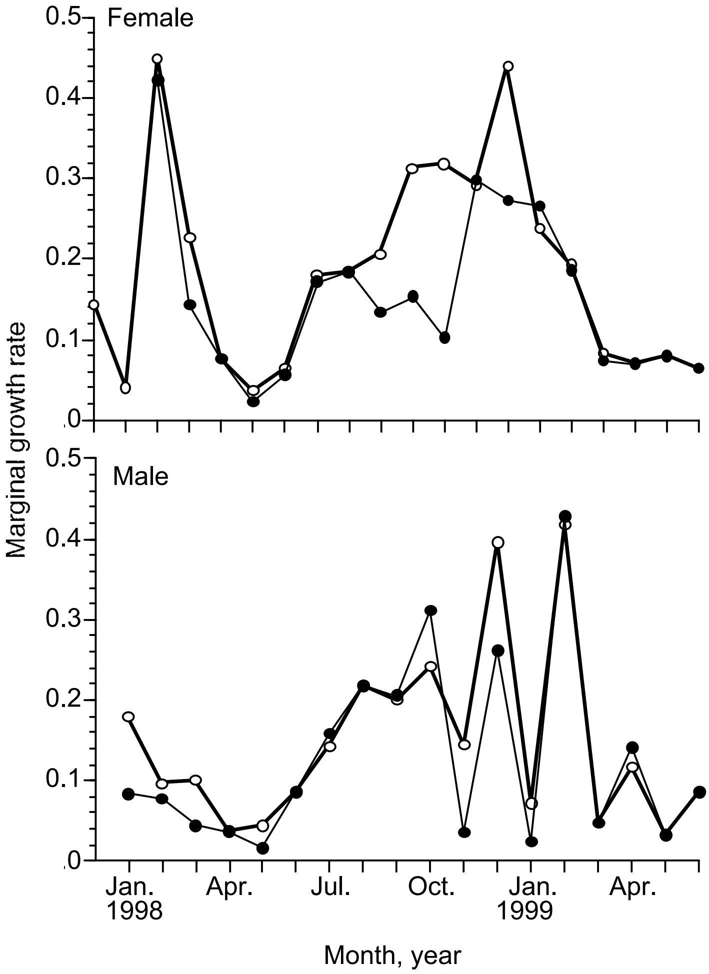

Figure 3. Marginal growth rate of otolith of turbot in the Black Sea. Open and closed circles indicate marginal growth rates if the thick and thin opaque zones, and the thick opaque zone only are regarded as the otolith ring, respectively. Top, female; bottom, male.

lowest marginal growth rates were recorded.

The open circles in Figure 3 indicate the marginal growth rate when the thick and thin opaque zones were considered as the otolith ring. The marginal growth rates of both sexes showed the same tendency from February to August as seen above. However, the marginal growth rate again decreased from August to October for both sexes. This suggests that the opaque zone was formed twice a year around May and October. Thus, we concluded that the thick opaque zone was considered as an annual otolith ring in this species. Accordingly, the ring radius was defined as the distance from the nucleus to the outer edge of each thick opaque zone, r_i as shown in Figure 1. In this study, we regarded the thin opaque zones as ring-like structures or sub-annual rings.

Figure 4 indicates the relations of the ring radius to otolith radius for selected ring groups in female. We observed 11 otolith rings at the maximum in female, although observation was limited for the 1, 6, 7, 8 and 9 year-old groups. The radius of each ring grew linearly with otolith radius for all ring groups, although we could not find the linear relation of the first ring radius to the otolith radius in the 5 ring group. Moreover, the variance for the same ring radius increased with ring group. The ring radius variance decreased with ring number. The relations of the ring radius to otolith radius in male are also shown in Figure 4 for the 2 to 5 ring groups only, because the number of otolith was small for other ring group. Although male data was few relative to that for female, we found the same relation of ring radius to otolith radius as female. Accordingly, these phenomena suggest that the growth of the ring radius was proportional to that of otolith.

Tables 2 and 3 show the mean ring radius of each ring in female and male, respectively. We found that the first, second, third and fourth ring radii

Table 2. Average radius r_i(mm) and back-calculated standard length $L(t)$(cm) at ring formation of female turbot in the Black Sea

Sample size	Ring group	Average radius(mm)										
		r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}	r_{11}
1	1	2.00										
101	2	1.89	4.06									
108	3	1.77	3.81	5.11								
45	4	1.39	3.22	4.77	5.68							
20	5	1.37	3.44	4.78	5.77	6.57						
28	6	1.56	3.34	4.58	5.44	6.13	6.71					
18	7	1.59	3.46	4.75	5.66	6.39	6.89	7.31				
6	8	1.42	3.07	3.92	4.58	5.22	5.70	6.17	6.58			
0	9											
1	10	2.30	3.70	5.00	6.00	6.50	7.10	7.70	8.00	8.30	8.60	
2	11	1.40	2.90	3.90	5.00	5.80	6.15	6.50	6.85	7.15	7.50	7.75
	Average	1.67	3.44	4.60	5.45	6.10	6.51	6.92	7.14	7.73	8.05	7.75
	$L(t)$	21.9	34.9	41.4	45.0	50.6	51.5	55.3	50.7	61.5	63.3	58.0

Table 3. Average radius r_i(mm) and back-calculated standard length $L(t)$ (cm) at ring formation of male turbot in the Black Sea

Sample size	Ring group	Average radius(mm)								
		r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9
2	1	1.50								
147	2	1.82	3.93							
121	3	1.69	3.63	4.86						
24	4	1.43	3.11	4.72	5.61					
9	5	1.44	2.89	3.86	4.83	5.64				
1	6	1.30	3.40	4.30	5.00	5.50	5.80			
6	7	1.67	3.33	4.38	5.12	5.68	6.12	6.48		
0	8									
1	9	1.60	3.80	5.50	6.40	7.00	7.40	7.70	8.10	8.30
Total, 311	Average	1.56	3.44	4.60	5.39	5.96	6.44	7.09	8.10	8.30
	$L(t)$	18.2	31.8	37.0	41.3	41.5	42.3	46.2	55.2	56.4

diminished with number of rings in both female (except r_4) and male. This condition, called the "Lee phenomenon", resulted in a necessary correction of the average radius. Thus, in this study, the outer ring radius of each ring group was used as the corrected ring radius.

Growth curve

The standard length L increased linearly with otolith radius R for both female and male at the significant level of 1%, $p < 0.01$ (Figure 5). The relation of L to R of each sex was represented by;

$$L = a + bR \quad (6)$$

where a (cm) and b are the intercept of L and the slope of L to R, respectively. The estimates of a and b were 9.40 and 6.27, respectively for female (coefficient of determination, 0.733), and 9.75 and 5.62, respectively, for male (coefficient of determination, 0.680). The slopes of L to R significantly differed between sexes ($p < 0.05$).

The back-calculated standard length was obtained from the corrected ring radius using Eq. (6), which gives the standard length at the time of the ring formation. Tables 2 and 3 summarize the average ring radii, back-calculated standard lengths, and number of samples of each ring group for female and male.

The Bertalanffy growth equations were determined from the back-calculated standard length at age of both female and male turbot. Table 4 shows the estimates of k, L_∞ and t_0, and their standard errors evaluated using the bootstrap method. The confidence limits of L_∞ and k were relatively narrow for both sexes. Moreover, Figure 6 indicates the relations of

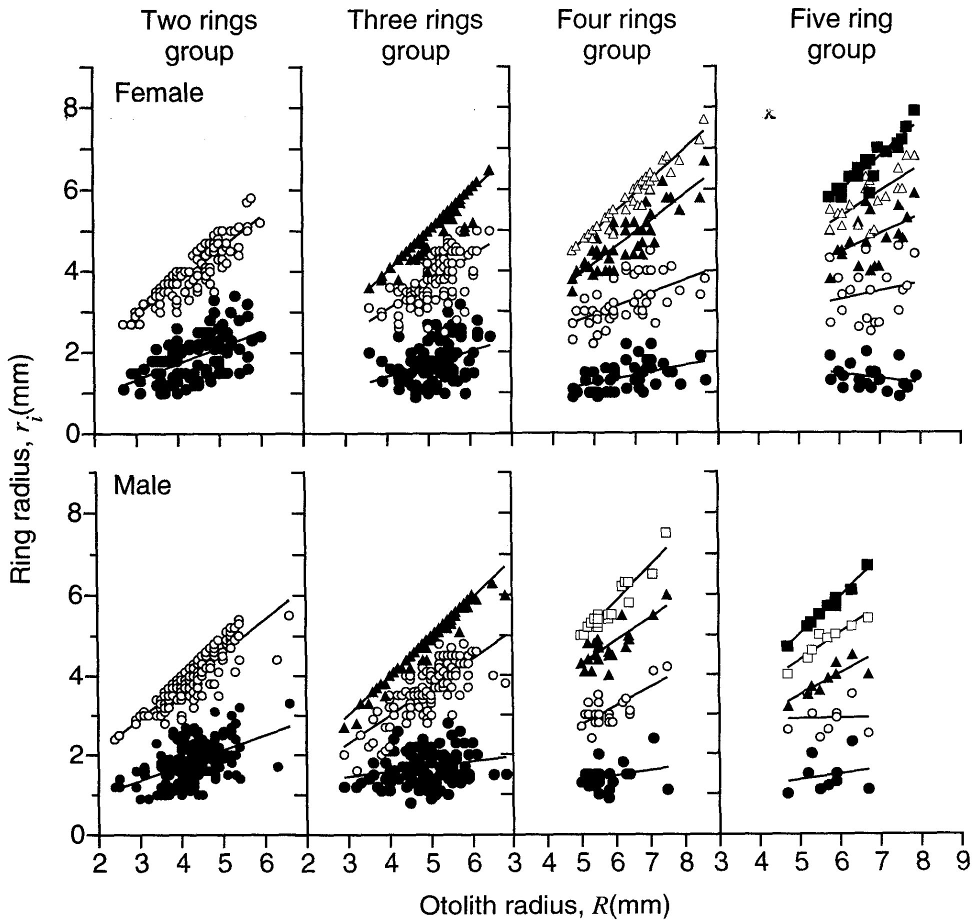

Figure 4. Relation of the ring radius ri(mm) to the otolith radius R(mm) of each ring group. Lines show the regression lines of r_i of each ring to R. ●, r_1; ○, r_2; ▲, r_3; △, r_4; ■, r_5. Top, female; bottom, male.

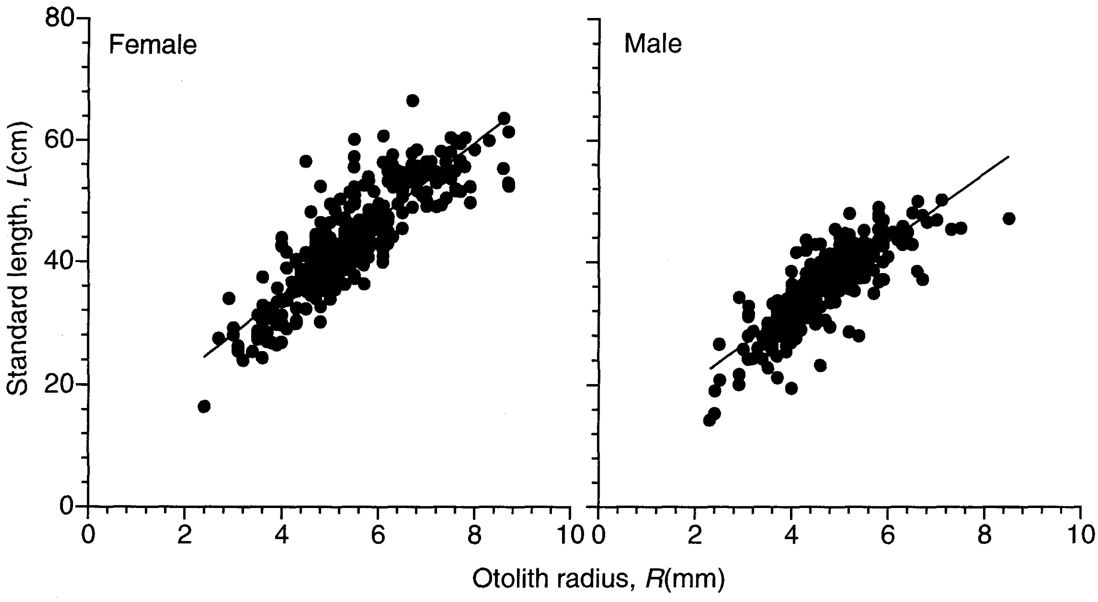

Figure 5. Relation of the standard length L(cm) to the otolith radius R(mm). Line indicates the regression line of L to R. Left, female; Right, male.

Table 4. Estimates of L_∞, k and t_0 of female and male turbot in the Black Sea, Gulf of Pomeranian and Gulf of Lion

Female	L_∞(cm)	k(year^{-1})	t_0(year)	R^2
Black Sea	54.8(0.350)	0.481(0.007)	-0.011	0.959
Gulf of Pomerania	51.9	0.200	0.296	
Gulf of Lion	54.6	0.307	-0.120	
Male				
Black Sea	45.0(0.539)	0.597(0.017)	-0.011	0.953
Gulf of Pomerania	33.4	0.347	0.413	
Gulf of Lion	54.4	0.235	-0.220	

Value in parentheses indicates standard error of each estimate.
R^2 is corrected coefficient of determination.

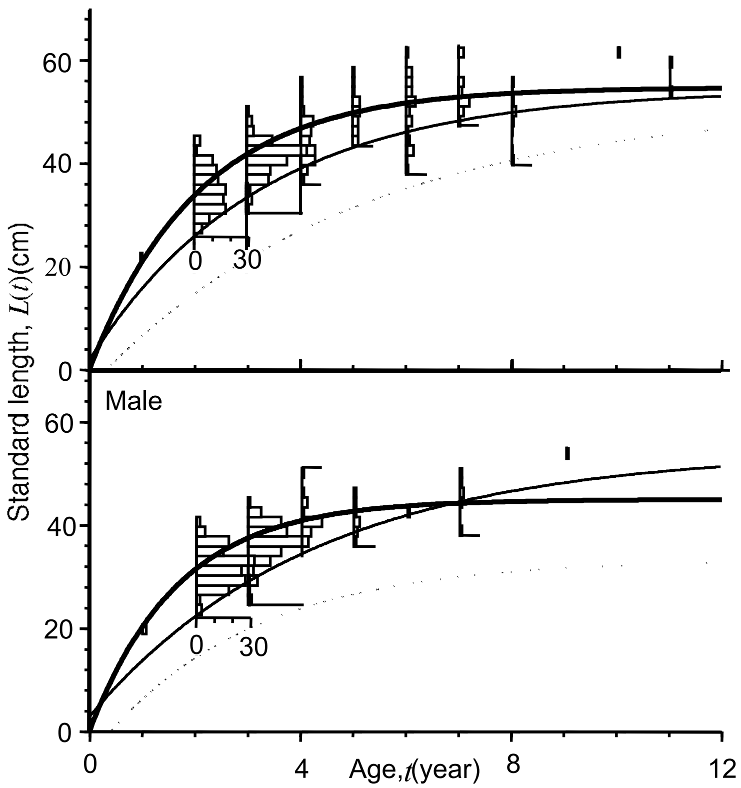

Figure 6. Relation of the standard length $L(t)$(cm) to age t(year). Thick, thin and dotted lines represent the Bertalanffy growth curves estimated in the present study, Robert and Vianet (1988), and Szlakowsky (1990), respectively. Top, female; bottom, male.

the back-calculated standard length to age of both sexes. The Bertalanffy growth curves convincingly explained the growth of turbot in the Black Sea. The both female and male turbot grew rapidly in length until 2 years old. The growth rate of male remarkably slowed after 2 years old. In female, the growth rate gradually decreased from 2 years old. However, the estimated curve did not closely match the back-calculated standard length at 8 and 9 years old, due to a few samples obtained of 8 and 9 years old for each sex.

Since there was no significant difference in the slope of standard length to total length between female and male ($p < 0.05$), the relation of standard length L to total length L' of each sex could be represented by

$$L = 0.804\, L', \tag{7}$$

where the coefficient of determination was 0.984. Using the non-linear regression method, from Eqs. (5) and (7), k of the released turbot was estimated to be 0.508 year^{-1} (L_∞ was assumed as 50.0 cm which was the weighted average of L_∞ given from the estimates of L_∞ for both sexes in the Black Sea).

Discussion

This study examined the relation of the length to age in turbot determined from the number of otolith rings. Beckman and Wilson (1995) reported that the period in which the opaque zone is formed is from spring to early summer for 60 to 70% of the fishes which range over the north temperate zone (from 23° to 45° N.). However, some fishes exist, of which the formative period of the opaque zone is from autumn to winter. Furthermore, they stated that the period of the opaque zone formation ranges from early spring to autumn in the sub arctic zone (located higher than 35° N.). In the Black Sea, however, the periods of opaque and hyaline zone formation in turbot were observed from February to May (late winter and spring) and from July to September (mid to late summer and early autumn), respectively. The opaque and hyaline zones in turbot in the Gulf of Lion (Mediterranean Sea) are formed during winter and summer, respectively (Robert and Viane, 1988). Additionally, Hass and Recksiek (1995), Tominaga *et al*. (1995), Solomon et al. (1987) and Yabuki (1989) reported that the opaque zones of winter flounder *Pleuronectes americanus*, pointhead flounder *Hippoglossoides pinetorum* pleuronectid flounder *Limanda yokohamae* and willowy flounder *Tanakius kitaharai* are formed in the period from February to May or June. These facts suggest that it is not unusual for the opaque zone to be formed in the period from February to May in Black Sea turbot. We cannot, however, show whether or not the period in which the opaque zone is formed corresponds to the growth period. This question should be examined in order to consider the mechanisms of opaque and hyaline zone formation.

Table 4 presents the estimates of the Bertalanffy growth equation, L_∞, k and t_0, of turbot in the Black Sea (this study), the Gulf of Pomerania (Szlakowski, 1990) and the Gulf of Lion (Robert and Viane, 1988). In addition, Figure 6 compares growth curves in three regions. The estimates of L_∞ in female were similar to those in the Black Sea, Gulf of Pomerania and Gulf of Lion. On the other hand, the estimates of L_∞ varied among three areas for male.

Moreover, the estimates of k in female turbot were 0.481, 0.200 and 0.307 year^{-1} in the Black Sea, Gulf of Pomerania and Gulf of Lion, respectively. On the other hand, the estimates of k in male turbot were 0.597, 0.347 and 0.235 year^{-1} in the Black Sea, Gulf of Pomerania and Gulf of Lion, respectively. The estimates of k for the Gulf of Pomerania appear to be very smaller than those in other regions especially for female. This fact suggests that the number of otolith rings has been miscounted in turbot of the Gulf of Pomerania, since the growth rates were similar to those of Black Sea turbot and the estimates of t_0 were positive for both sexes. For example, if the number of otolith rings in Gulf of Pomerania specimens was twice as many as that in other regions, the estimates of k could be calculated as 0.400 and 0.694 for female and male, respectively. Such hypothetical estimates are in fact similar to those in the present study. Consequently, we concluded that the age of turbot in the Gulf of Pomerania has likely been over-estimated.

Finally, let us compare the estimate of k from specimens obtained from the commercial fisheries and the net survey, and that calculated from the tag-recapture data in Black Sea turbot. The estimate of k from the specimens of the released turbot was 0.508 year^{-1}. This estimate is very similar to the weighted mean of k in female and male, which was 0.537 year^{-1}. This indicates that the present study correctly carried out the counting of otolith rings and hence age determination.

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