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# Age and Growth of Marbled Sole *Pleuronectes yokohamae* in Kikonai Bay, Hokkaido, Japan

**Mohamad M. Shafieipour,[1,*] Toyomi Takahashi,[1] Kengo Ishino,[2] Tetsuya Takatsu,[1] and Toshikuni Nakatani[1]**

1) Faculty of Fisheries, Hokkaido University, Minato, Hakodate, Hokkaido 041–0821, Japan
2) Hokkaido Hakodate Fisheries Experimental Station, Yunokawa, Hakodate, Hokkaido 042–0932, Japan

**Abstract:** Age and growth of marbled sole *Pleuronectes yokohamae* were estimated from growth increments in otolith. The samples were collected with commercial set nets and an experimental bottom trawl net in Kikonai Bay and the nearby waters, southern coast of Hokkaido, from May 1994 to July 1995. Monthly changes in the percentage occurrence of otoliths with translucent edge in one hand and the marginal increments on the other hand verified that the translucent zone was formed once a year after their spawning season in May, indicating availability of outer margin of translucent zone as an annulus. The regression method as well as Fraser–Lee's method were used to estimate the back–calculated lengths. Growth of marbled sole was expressed by the von Bertalanffy asymptotic growth function: $TL_t=361.78[1-\exp^{-0.390(t-0.098)}]$ for males and $TL_t=438.15[1-\exp^{-0.323(t-0.145)}]$ for females using back–calculated lengths by the regression method and $TL_t=367.04[1-\exp^{-0.388(t-0.114)}]$ for males and $TL_t=436.05[1-\exp^{-0.323(t-0.147)}]$ for females by the Fraser–Lee's method, where $TL_t$ is the total length (millimeters) at estimated age t. The theoretical lengths predicted by these two methods at all estimated ages were very similar. It was found that the growth rate of marbled sole in this study area was relatively high compared to those obtained in the Inland Sea of Japan as. However, the highest growth rate was observed with regard to the population off Fukushima Prefecture, although the bottom water temperatures were similar to those in Kikonai Bay. It is inferred that the geographical variation in the growth rates of marbled sole is influenced by the hydrographic and food conditions.

**KEY WORDS:** age, growth, otolith, marbled sole, geographical variation

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[*] Present address: Iranian Fisheries Research Organization, # 24, 11th Alley, Mir-Emad St., Mothahari Ave., Tehran, Iran. P.O. Box: 14155-6116, Tel: +9821-8751498-99; Fax: +9821-8751495; e-mail:ifrto@dci.iran.com

## Introduction

Marbled sole *Pleuronectes yokohamae* is distributed in the coastal waters around Japan from southern Hokkaido to Oita Prefecture in Kyushu, the Yellow Sea, and the northern part of the East China Sea. This species, which is an important commercial fish in Japan, inhabits sandy–mud bottoms shallower than 100m depths (Sakamoto, 1984), and is mainly caught with gill nets and set nets. Annual landings of marbled sole in Kikonai Bay and the adjacent waters (from Fukushima to Hakodate) between 1985 to 1995 ranged from 173 to 349 metric tons (Government of Hokkaido, 1996). Hatanaka and Iwashi (1953) determined the age of marbled sole using the scales as well as the otoliths, however they discovered that using otoliths were more conspicuous and more satisfactory way to determine the age. Thereafter the age and growth of marbled sole has been studied in the Seto Inland Sea, (Toyama, 1974; Matsumura *et al.,* 1974; Masaki *et al.* 1986; Tanda *et al.,* 1992), Ise Bay (Suzuki, 1967), Tokyo Bay (Solomon *et al.,* 1987), and the coastal waters of Fukushima Prefecture (Fukushima Pref. Fish. Exp. Sta. 1987), but there is no information available about the age and growth of this fish in Hokkaido. The object of this study was to estimate the age and growth rate using otoliths of marbled sole in Kikonai Bay and the nearby waters, southern coast of Hokkaido, and to compare the growth rates of several populations around Japan.

## Materials and Methods

The samples were collected from Kikonai Bay and the nearby waters, southern Hokkaido, from May 1994 to July 1995 (Table 1, Fig. 1).

Most of specimens (90%) were caught with commercial set nets. Remaining fish were obtained with commercial gill nets and an experimental bottom trawl net. The total lengths of fish were measured to the nearest millimeter, and their sex was also recorded. The paired otoliths were removed and kept dry. The otolith growth of Pleuronectid fish is faster on the axis of the ocular side extending the anterior part of the body (Wada, 1970), revealing the applicability of ocular side of the otolith for reading purposes, especially with respect to the older fishes. Therefore, only the ocular side otolith was used for age determination and growth analysis. After being submerged in the water for several hours, the concave side of all the otoliths were ground with fine sandpaper (# 600 grits) so that the polished edge passed through the focus of otolith. The ground otoliths were mounted on glass slides coated with salad oil, which were placed against a black background to improve the visibility. The 20 times magnified image of the otoliths were then viewed using a binocular microscope. The otolith radius (R), to the anterior margin, and the ring radius ($r_n$) to each translucent margin were measured to the nearest 0.05 mm along the longest axis (Fig. 2). In order to determine the annulus formation time, a seasonal changes in the percentage occurrence of the otoliths with translucent edges was examined. Seasonal changes in marginal increments of the otoliths (MI) were also calculated to examine the growth pattern of the otoliths:

$$MI = (R - r_n) / (r_n - r_{n-1}) \qquad (1)$$

where R is the otolith radius (mm) and $r_n$ is the distance (mm) between the focus and outer margin of the last translucent zone.

**Table 1.** List of marbled sole analyzed in this study

| Sampling date | Sampling gear | Number of individuals (Range TL, mm) | | |
|---|---|---|---|---|
| | | Male | Female | Total |
| 17 May 1994 | Set net | 28 (204-344) | 43 (198-422) | 71 |
| 15 June 1994 | Set net | 4 (311-335) | 25 (307-379) | 29 |
| 29 June 1994 | Set net | 41 (193-340) | 65 (193-393) | 106 |
| 21 July 1994 | Set net | 13 (177-362) | 22 (173-450) | 35 |
| 8 Aug. 1994 | Set net | 58 (167-288) | 51 (169-294) | 109 |
| 12 Sep. 1994 | Set net | 36 (182-243) | 45 (178-228) | 81 |
| 21 Sep. 1994 | Set net | 40 (215-337) | 50 (219-411) | 90 |
| 12 Oct. 1994 | Set net | 11 (169-340) | 34 (188-464) | 45 |
| 25 Oct. 1994 | Set net | 26 (183-314) | 31 (185-362) | 57 |
| 14 Nov. 1994 | Set net | 27 (215-336) | 34 (213-416) | 61 |
| 21 Nov. 1994 | Set net | 44 (177-314) | 32 (185-318) | 76 |
| 7 Dec. 1994 | Set net | 37 (194-342) | 30 (186-448) | 67 |
| 24 Jan. 1995 | Set net | 27 (206-391) | 44 (214-462) | 71 |
| 24 Feb. 1995 | Set net | 27 (208-332) | 29 (272-407) | 56 |
| 6 Mar. 1995 | Set net | 32 (180-329) | 26 (195-428) | 58 |
| 5 Apr. 1995 | Gill net* | 32 (110-324) | 49 (208-449) | 81 |
| 26 Apr. 1995 | Set net | 39 (175-309) | 63 (181-431) | 102 |
| 15 May 1995 | Set net | 52 (162-350) | 90 (166-444) | 142 |
| 26 June 1995 | Set net | 52 (179-333) | 67 (173-386) | 119 |
| 5 July 1995 | Set net | 9 (235-334) | 24 (220-451) | 33 |
| 17 July 1995 | Bottom trawl | 36 (91-177) | 44 (101-177) | 80 |
| 19 July 1995 | Set net | 40 (176-340) | 67 (157-446) | 107 |
| Total | | 711 (91-350) | 965 (101-464) | 1,676 |

* These data were used only for annulus formation time analysis.

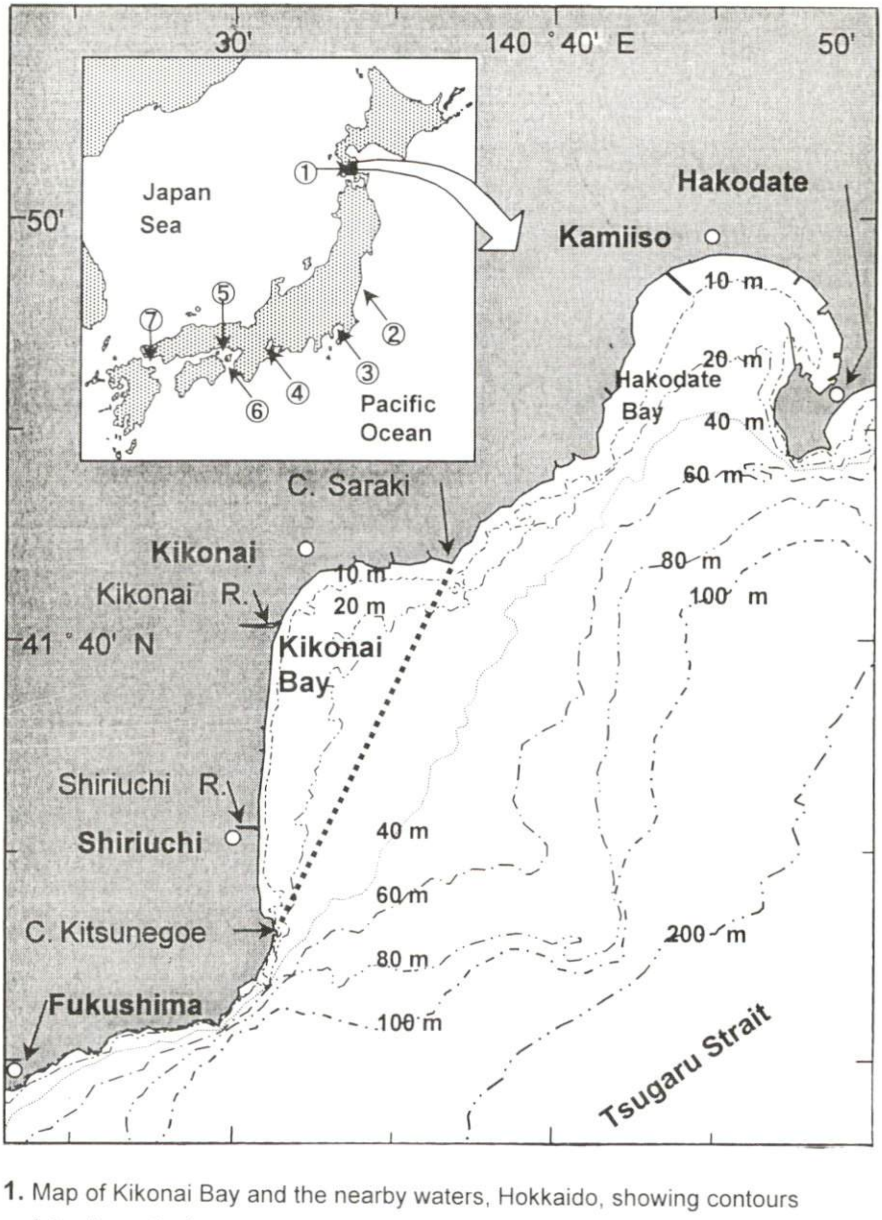

**Fig. 1.** Map of Kikonai Bay and the nearby waters, Hokkaido, showing contours of depth, and other areas referred in the text.
① : Kikonai Bay; ② : Fukushima Prefecture; ③ : Tokyo Bay;
④ : Ise Bay; ⑤ : Harima-Nada; ⑥ : Kii Channel;
⑦ : Suō–Nada

The Regression method and Fraser–Lee's method (Carlander, 1981) were applied to estimate the back-calculated total lengths ($TL_n$) at the formation time of the outer margins of the translucent zone:

$$TL_n = br_n + d \qquad (2)$$

where b and d are the slope and intercept of the regression, respectively. Fraser–Lee's equation is expressed as follows:

$$TL_n = d + (TL_c - d) \times (r_n / R) \qquad (3)$$

where $TL_c$ is the total length at capture.

## Results

*Formation of translucent zone*

Because there was no significant difference in the ratio of the otoliths with translucent edges between sexes except in 17 May 1994 (chi–square test, $P < 0.05$), both sexes were combined in Fig. 3. All the otoliths from September to March had translucent edges. After April, the percentage occurrence of the otoliths with translucent edges began to decrease, indicating the opaque zone is being formed. The minimum value was registered in late June, while it was increasing from July to September. These results show that the outer margin of translucent zone is formed from April to June, mainly during May. The spawning of marbled sole in this study area takes place from February to April (Government of Hokkaido, 1995), indicating the time of formation of the translucent margin approximately coincides with the end of the spawning season.

*Seasonal changes in growth of the otolith*

Seasonal change in marginal increments of otoliths (MI) indicated that a translucent zone was formed once a year (Fig. 4).

Minimum values of marginal increments for both sexes were observed in April. These values then increased gradually, and reached to the maximum approximately in the next April. Relatively high values were also found in May for males and from May to July for females, but these fishes with large translucent zone were collected in small numbers.

*Relationship between total length and otolith radius*

Fig. 5, illustrate the approximately linear relationship between otolith radius (R) and total length (TL). These values and the least-squares equation were expressed as follows:

$$\text{Males: } TL = 85.25\,R - 18.53, \qquad (n = 675,\ r^2 = 0.880) \qquad (4)$$

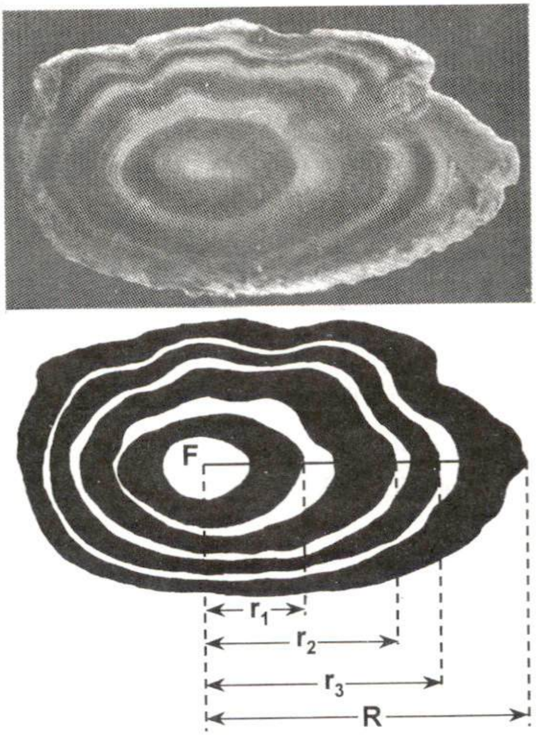

**Fig. 2.** Ocular side otolith of marbled sole with three annuli (male, 264 mm in total length)
F: focus; R: otolith radius; $r_1$~$r_3$ : annual ring radii

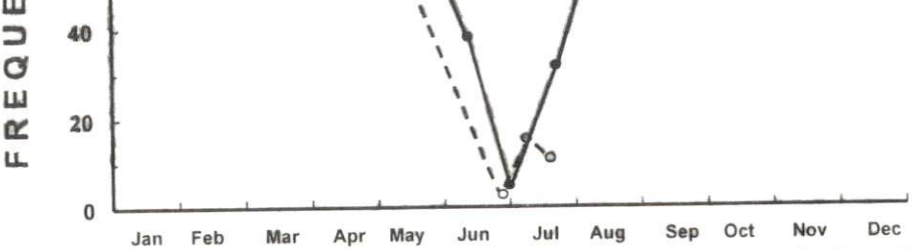

**Fig. 3.** Monthly change in percentage of occurrences of otoliths with translucent edge for marbled sole in 1994 (closed circles) and 1995 (open circles). Both sexes were combined

Females: $TL = 93.37 R - 35.52$, $(n = 915, r^2 = 0.900)$ (5)

The residuals and slope of the regression line were significantly different between sexes at the 1% level (ANCOVA). Therefore, the data were treated separately for each sex. The mean ring radii (distances from the focus to the successive translucent margin) are shown in Table 2. These results demonstrated that, a clear Lee's or reverse–Lee's phenomenon was not found for either sex. No significant difference between sexes was found in the mean values of $r_1$–$r_2$, while the mean values of $r_3$–$r_6$ of females were significantly larger than those of males (t–test, $P < 0.01$).

The estimated total lengths at the formation of translucent margin from the equation (2) and Fraser–Lee's method equation (3) were similar (Table 3).

Walford's growth transformations (Warlford, 1946) of mean back–calculated length were as follows:

Regression method:

Males: $TL_{n+1} = 0.676\ TL_n + 117.26$; $TL_\infty = 361.78$, $(r^2 = 0.964)$ (6)

Females: $TL_{n+1} = 0.722\ TL_n + 121.73$; $TL_\infty = 438.15$ $(r^2 = 0.997)$ (7)

Fraser–Lee's method:

Males: $TL_{n+1} = 0.677\ TL_n + 118.48$; $TL_\infty = 367.04$ $(r^2 = 0.961)$ (8)

Females: $TL_{n+1} = 0.715\ TL_n + 124.32$; $TL_\infty = 436.04$ $(r^2 = 0.997)$ (9)

Where $TL_\infty$ is the total length (mm) at infinite age, and $TL_n$ is the total length (mm) at the formation of translucent margin. From these regressions, von Bertalanffy growth equations (von Bertalanffy, 1937) were calculated;

Regression method:

Males: $TL_t = 361.78\ [1 - \exp^{-0.390(t - 0.098)}]$ (10)

Females: $TL_t = 438.15\ [1 - \exp^{-0.323(t - 0.145)}]$ (11)

Fraser –Lee's method:

**Males:** $TL_t = 367.04\ [1 - \exp^{-0.388(t - 0.114)}]$ (12)

**Females:** $TL_t = 436.05\ [1 - \exp^{-0.323(t - 0.147)}]$ (13)

Where $TL_t$ is the **total length (mm) at the** formation of **translucent** margin. Theoretical lengths for both sexes estimated by the two back–calculated methods were quite similar (Table 4, Fig. 6).

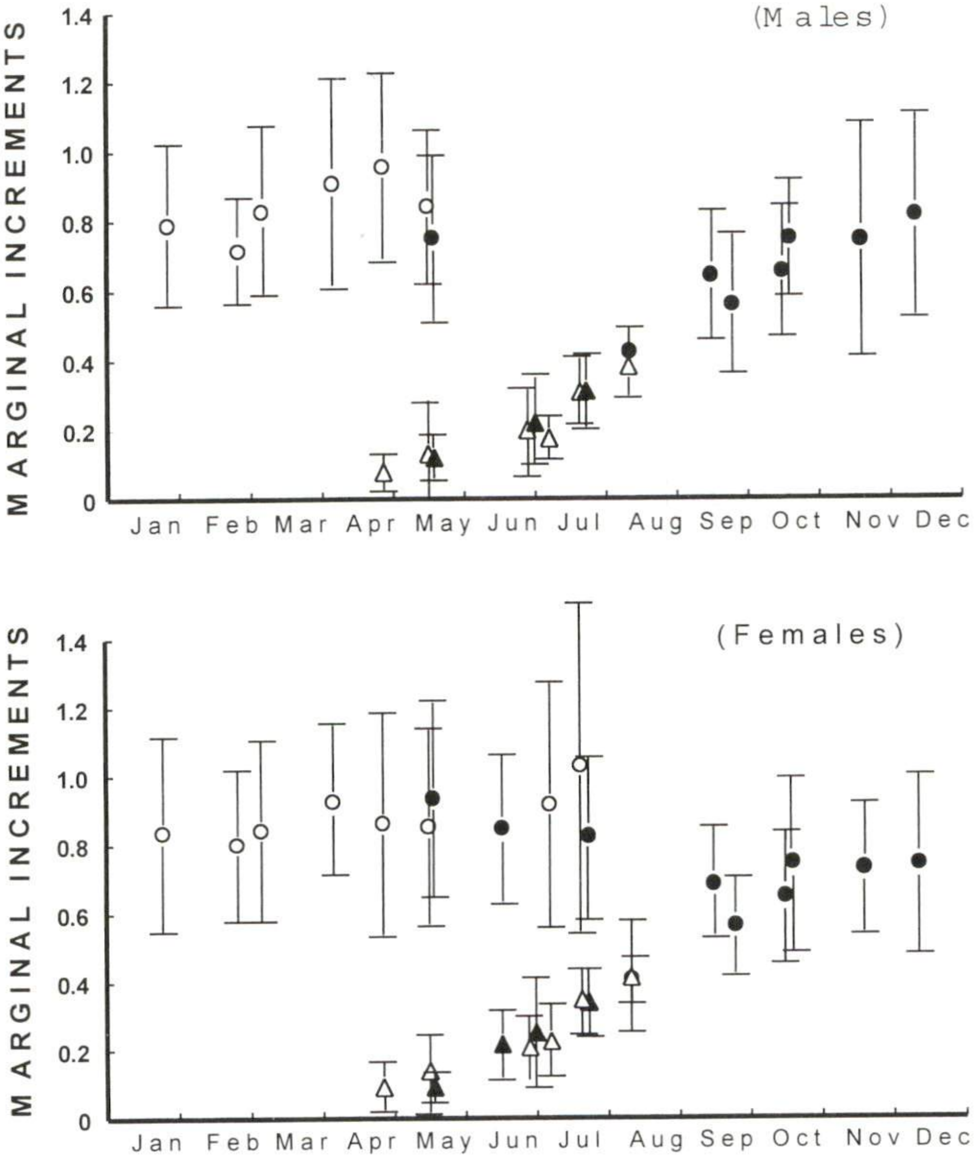

**Fig. 4.** Monthly changes in marginal increments of otoliths of marbled sole. Circles and triangles indicate the mean values of marginal increments of otoliths with translucent edge and opaque edge, respectively. Closed and open marks indicate the samples in 1994 and 1995, respectively. Vertical bars indicate standard deviations.

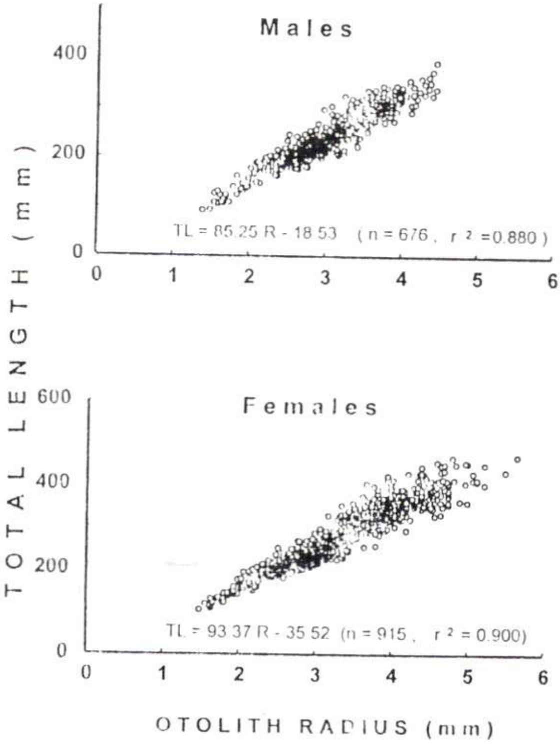

**Fig. 5.** Relationships between otolith radius and total length for male and female marbled sole.

**Table 2**. Mean ring radii (±SD) for each ring group of marbled sole

| Ring groups | N | R | Ring radii (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $r_1$ | $r_2$ | $r_3$ | $r_4$ | $r_5$ | $r_6$ | $r_7$ |
| Male | | | | | | | | | |
| I | 237 | 2.61 ± 0.37 | 1.54 ± 0.20 | | | | | | |
| II | 248 | 2.97 ± 0.32 | 1.51 ± 0.23 | 2.53 ± 0.30 | | | | | |
| III | 143 | 3.46 ± 0.34 | 1.50 ± 0.24 | 2.48 ± 0.27 | 3.09 ± 0.30 | | | | |
| IV | 31 | 3.82 ± 0.31 | 1.48 ± 0.21 | 2.46 ± 0.27 | 3.05 ± 0.32 | 3.61 ± 0.27 | | | |
| V | 13 | 4.01 ± 0.20 | 1.40 ± 0.23 | 2.43 ± 0.30 | 3.01 ± 0.28 | 3.48 ± 0.22 | 3.69 ± 0.20 | | |
| VI | 7 | 4.24 ± 0.18 | 1.36 ± 0.18 | 2.40 ± 0.28 | 2.92 ± 0.22 | 3.55 ± 0.23 | 3.73 ± 0.17 | 4.09 ± 0.14 | |
| Weighted mean | 679 | | 1.51 ± 0.22 | 2.50 ± 0.29 | 3.07 ± 0.27 | 3.57 ± 0.24 | 3.70 ± 0.19 | 4.09 ± 0.14 | |
| Female | | | | | | | | | |
| I | 217 | 2.62 ± 0.49 | 1.54 ± 0.23 | | | | | | |
| II | 289 | 3.04 ± 0.34 | 1.52 ± 0.21 | 2.57 ± 0.28 | | | | | |
| III | 242 | 3.71 ± 0.39 | 1.52 ± 0.23 | 2.53 ± 0.27 | 3.26 ± 0.32 | | | | |
| IV | 73 | 4.01 ± 0.38 | 1.48 ± 0.22 | 2.49 ± 0.32 | 3.21 ± 0.35 | 3.73 ± 0.40 | | | |
| V | 46 | 4.32 ± 0.40 | 1.54 ± 0.25 | 2.48 ± 0.32 | 3.19 ± 0.33 | 3.72 ± 0.36 | 4.09 ± 0.38 | | |
| VI | 37 | 4.53 ± 0.41 | 1.46 ± 0.26 | 2.47 ± 0.31 | 3.13 ± 0.37 | 3.66 ± 0.38 | 4.04 ± 0.40 | 4.32 ± 0.42 | |
| VII | 11 | 4.69 ± 0.40 | 1.50 ± 0.25 | 2.52 ± 0.37 | 3.21 ± 0.42 | 3.69 ± 0.41 | 4.05 ± 0.38 | 4.33 ± 0.39 | 4.59 ± 0.39 |
| Weighted mean | 915 | | 1.52 ± 0.24 | 2.54 ± 0.29 | 3.23 ± 0.39 | 3.71 ± 0.38 | 4.07 ± 0.38 | 4.32 ± 0.41 | 4.59 ± 0.39 |

N: number of otoliths used.

**Table 3.** Mean back-calculated total length (mm) at the time of each annulus formation

| | $TL_1$ | $TL_2$ | $TL_3$ | $TL_4$ | $TL_5$ | $TL_6$ | $TL_7$ |
|---|---|---|---|---|---|---|---|
| Regression | | | | | | | |
| Male | 110.3 (90.7) | 194.5 (161.4) | 242.8 (202.0) | 286.03 (238.3) | 297.1 (247.5) | 330.1 (275.3) | |
| Female | 106.5 (87.7) | 201.2 (167.2) | 265.9 (221.5) | 310.78 (259.2) | 344.1 (287.2) | 367.8 (307.1) | 393.4 (328.6) |
| Fraser- Lee | | | | | | | |
| Male | 108.2 (88.9) | 192.1 (159.4) | 243.6 (202.6) | 293.8 (244.8) | 301.7 (251.4) | 332.8 (277.6) | |
| Female | 103.3 (85.0) | 195.3 (162.2) | 268.7 (223.9) | 318.1 (265.3) | 347.3 (289.9) | 372.4 (311.0) | 391.6 (327.1) |

Figures in parentheses are standard lengths (SL) converted using equations:

$SL=0.84TL-2.89$ ($r^2=0.989$, N= 544) for Male, $SL= 0.85TL-3.88$ ($r^2=0.982$, N=428) for female.

**Table 4.** Mean total length (mm) at the time of each annulus formation of marbled sole calculated from von Bertalanffy growth equations. (Reg.: back-calculation by regression method; Fraser-Lee: back-calculation by Fraser-Lee's method)

| | Male | | Female | |
|---|---|---|---|---|
| Age | Reg. | Fraser-Lee | Reg. | Fraser-Lee |
| 1 | 107.4 | 106.9 | 105.6 | 105.1 |
| 2 | 189.6 | 190.6 | 197.4 | 196.4 |
| 3 | 245.2 | 247.4 | 263.8 | 262.5 |
| 4 | 282.9 | 285.9 | 311.9 | 310.4 |
| 5 | 308.4 | 312.0 | 346.8 | 345.1 |
| 6 | 325.6 | 329.7 | 372.0 | 352.2 |
| 7 | | | 390.2 | 388.4 |

According to the analysis of covariance (ANCOVA), growth coefficients (k) of females estimated by two methods were significantly higher than those of males ($P < 0.01$).

## Discussion

As described before, the translucent zone of marbled sole otolith in Kikonai Bay was formed once a year, and the formation of the translucent margin occurred approximately by the end of the spawning season. Therefore, the outer margin of successive translucent zone indicates the annuli as the same as several populations of marbled sole.

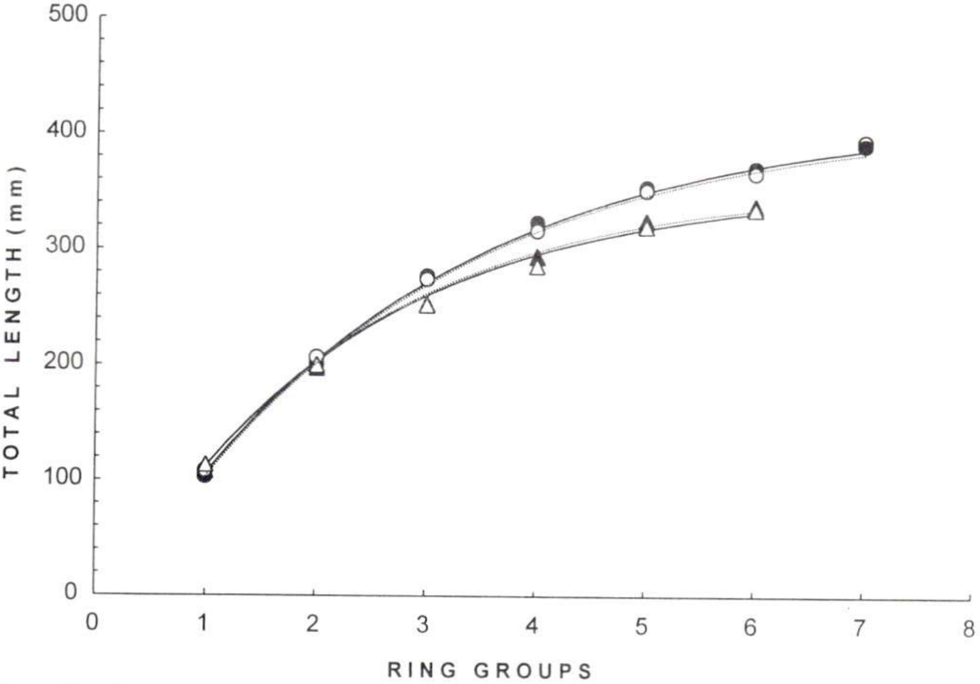

**Fig. 6.** Von Bertalanffy growth curves for marbled sole calculated by regression method (solid lines) and Fraser–Lee's method (dotted lines). The plots represent back–calculated total Length; open triangles: males by regression method; closed triangles: males by Fraser–Lee's method; open circles: females by regression method; closed circles: females by Fraser– Lee's method.

As shown in Fig. 7 and Fig. 8, the actual length of male and female young fishes caught with set nets were larger than the back–calculated length, however the length of the one– year– old fishes obtained with trawl net at the depth of 8–9 m correlated closely to von Bertalanffy growth curve.

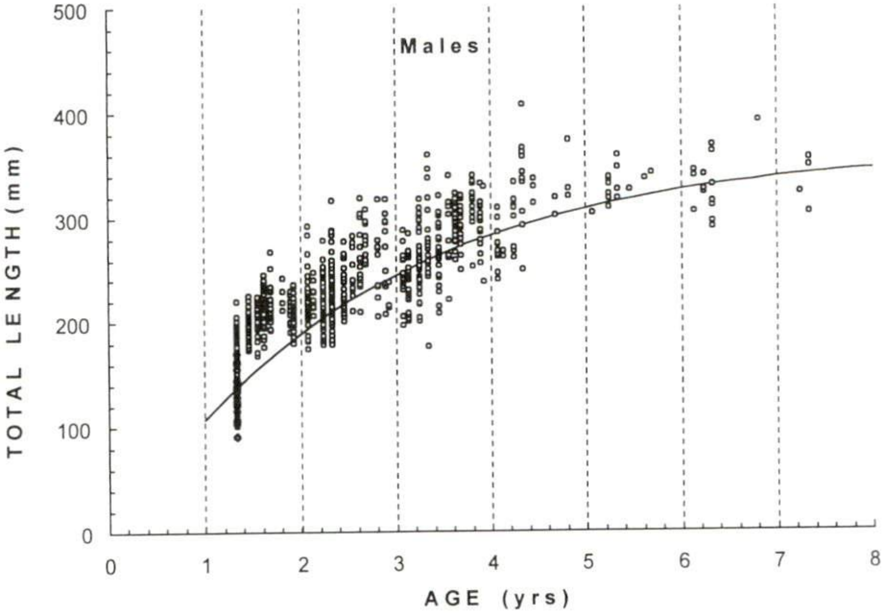

**Fig. 7.** Von Bertalanffy growth curve fitted to total length of male marbled sole. Circles and rhombi are fish taken from set nets and trawl net, respectively.

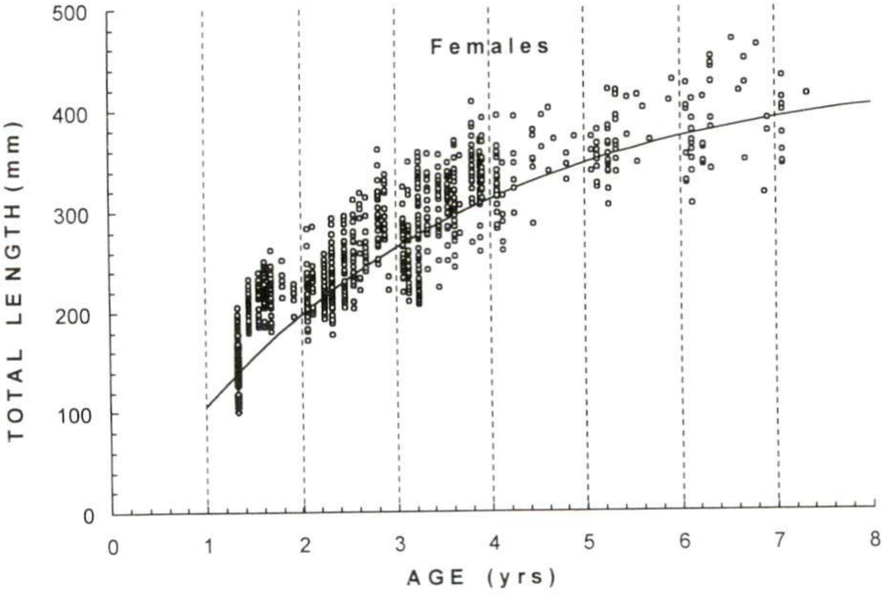

**Fig. 8.** Von Bertalanffy growth curve fitted to total length of female marbled sole. Circles and rhombi are fish caught by set nets and trawl net, respectively.

Since the set net fishing was operated at a depth more than 20 m (Fig. 1), we supposed that the fish younger than 2 years old with tardy growth gathered in shallower area (below 20 m).

According to Tanda *et al.*, (1992) the highest growth rate of marbled sole occurred in Tokyo Bay, while the growth rate in Harima–Nada, Kii Channel and Suō–Nada were rather lower.

Total lengths of marbled sole at different age and growth curves in the Japanese waters are shown in Table 5, Fig. 9 and Fig. 10.

**Table 5.** Comparison of growth (total length, mm) of marbled sole in coastal waters around Japan

| Estimated age | Kikonai Bay[1] | | Suō-Nada[2] | | Harima-Nada*[3] | | Tokyo Bay*[4] | | Kii Channel[5] | | Fukushima Pref.[6] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 1 | 107.4 | 105.6 | 114 | 111 | 126.5 | 127.5 | 132.9 | 146.9 | 124.0 | 120.3 | 180.0 | 173.3 |
| 2 | 189.6 | 197.4 | 176 | 182 | 187.1 | 201.1 | 206.6 | 224.3 | 187.1 | 195.2 | 221.9 | 243.9 |
| 3 | 245.2 | 263.8 | 217 | 235 | 231.9 | 258.1 | 250.8 | 281.0 | 223.4 | 246.3 | 257.8 | 303.6 |
| 4 | 282.9 | 311.9 | 243 | 273 | | 305.7 | 277.4 | 322.4 | 242.8 | 281.9 | 288.4 | 354.1 |
| 5 | 308.4 | 346.8 | 260 | 300 | | | 293.2 | 352.8 | 254.9 | 305.7 | 314.5 | 396.9 |
| 6 | 325.6 | 372.0 | 270 | 321 | | | | 375.2 | 261.0 | 322.3 | 336.9 | 433.0 |
| 7 | | 390.2 | | | | | | | | | 355.9 | 463.6 |
| 8 | | | | | | | | | | | 372.2 | 489.5 |
| 9 | | | | | | | | | | | | 511.4 |
| 10 | | | | | | | | | | | | 529.9 |
| $L_\infty$ | 361.8 | 438.2 | 290.8 | 378.2 | | 502.2 | 317.2 | 436.5 | 270.3 | 361.1 | 467.3 | 631.6 |

* Standard lengths were converted to total lengths using equations: TL= 1.212SL+1.64 for Male; TL= 1.188SL+5.10 for female.

[1] This study, [2] Masaki *et al.* (1986), [3] Matsumura *et al.* (1974), [4] Solomon *et al.* (1987), [5] Tanda *et al.* (1992), [6] Fukushima Fish. Exp. Sta. (1987).

As seen in Fig. 10, the highest growth rate was registered in connection with the female marbled sole population off Fukushima Prefecture, followed by populations inhibiting Tokyo Bay and Kikonai Bay.

On the other hand, the growth rates in Suō–Nada and Kii Channel in the Inland Sea were comparatively very low. A similar fact was observed with respect to male marbled sole (Fig. 9).

Kitagawa *et al.* (1994) and Takahashi *et al.* (1995) have studied the impact of geographical variations on the growth of flounders. Kitagawa *et al.* (1994) pointed out that due to low temperature the tardy growth season of Japanese flounder *Paralichthys olivaceus* is longer in the north of Japan.

Takahashi *et al.* (1995) have also reported that the bottom temperature appears to be an important factor for explaining the geographical variation in growth rate of brown sole *Pleuronectes herzensteini* in the northern coast of the Japan Sea.

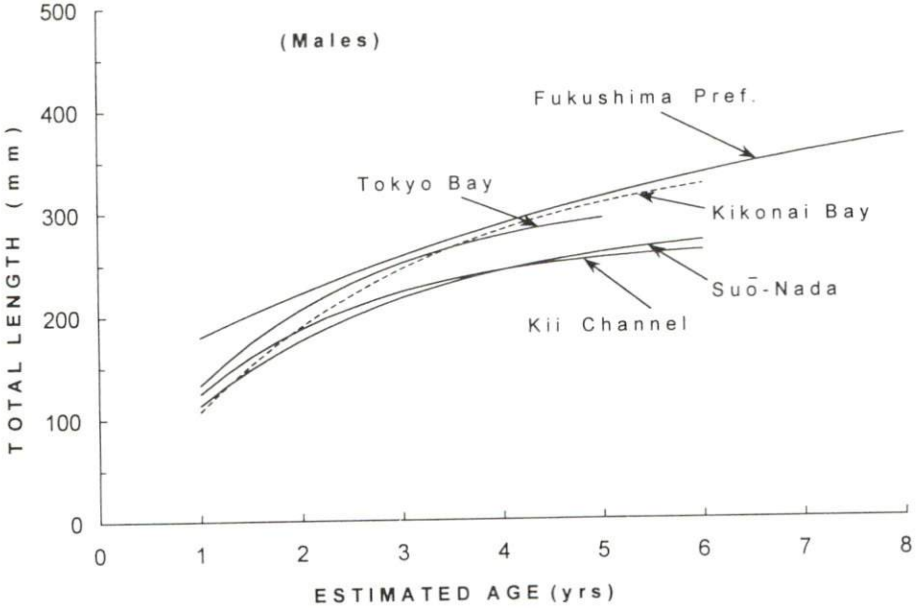

**Fig. 9.** Comparison of von Bertalanffy growth curves for male marbled sole in several areas around Japan (data from Harima-Nada was not sufficient for providing of curve).

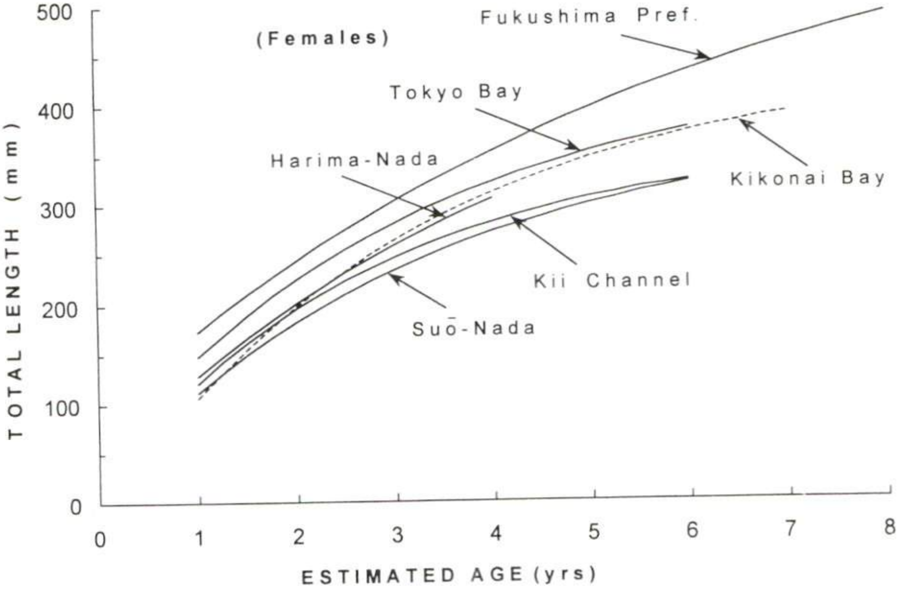

**Fig. 10.** Comparison of von Bertalanffy growth curves for female marbled sole in several areas around Japan.

According to our observation taken place from 1994 to 1998, the bottom water temperatures at the depth of 30m (the main fishing ground 20-40m depth) in Kikonai Bay ranged from 6.4℃ in March to 19.8℃ in October, except for a remarkably high temperature (22.3℃) in August 1994. While those at about the depth of 30m (fishing ground 25-69m, Hatanaka *et al.*, 1956) off Fukushima Prefecture from 1993 to 1994 ranged from 8.0℃ in April to 20.3℃ in November (Fukushima Fish. Exp. Sta., 1995). Thus, there is small difference in shallower water temperatures between these two sea area, especially during the feeding season. In contrast, the bottom temperatures in Kii Channel, which is situated in southern Japan, are high throughout the year. Namely, the temperatures ranged 14 to 16℃ in January and 23 to 24℃ in October (averaged values during the years 1967 to 1980; Horiki, 1992).

Takahashi *et al.* (1987) found that the daily feeding rate of marbled sole began to decrease at about 25℃ and the upper lethal limit lay in 28-30℃. The maintenance rations of daily food consumption of marbled sole increased from 1.3% at 8.4℃ to 1.8% at 19.0℃. According to these facts, low growth rates in Kii Channel and Suō–Nada (Fig. 9 and Fig. 10) may be due to high temperatures above the optimum temperatures for growth during the feeding season. Nevertheless, it cannot be considered that the difference of growth rates between Fukushima Prefecture and Kikonai Bay is due to water temperature, suggesting effects of other factors such as food condition.

To confirm the cause affecting the geographical variation in growth of marbled sole, It needs further information on the optimum temperature for the growth, hydrographic and food conditions in their habitats.

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