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Age and Growth of Pointhead Flounder *Hippoglossoides pinetorum* in Ishikari Bay, Hokkaido

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Pointhead flounder, *Hippoglossides pinetorum*, taken by commercial gillnets and an experimental beam trawl net in Ishikari Bay, Hokkaido from September 1991 to July 1993 were aged using polished otoliths (saggitae). Ages were validated by the monthly change in the percentage of otolith with translucent edge and marginal increments; a single opaque and translucent zone was formed annually. The regression method and Lee (Fraser-Lee)'s method were used to estimate the back-calculated length. Growth of pointhead flounder was expressed as $L_t=251.9(1-\exp^{-0.261(t-0.142)})$ for males and $L_t=315.1(1-\exp^{-0.219(t-0.200)})$ for females using back-calculated lengths by the regression method and $L_t=251.6(1-\exp^{-0.263(t-0.142)})$ for males and $L_t=333.0(1-\exp^{-0.204(t-0.186)})$ for females using back-calculated lengths by Lee's method, where L_t is the standard length (mm) at estimated age t. Small differences in the theoretical lengths of older female fish were found between the two methods. However, the theoretical standard lengths predicted by the two methods were very similar at most estimated ages and indicated the approximate actual length at each age.

Key words: *Hippoglossoides pinetorum*, age, growth, otolith

Pointhead flounder *Hippoglossoides pinetorum* is an important commercial fish off the coastal waters of Hokkaido and south-western Honshu Island. In the coastal waters of Hokkaido, the annual catch of this species by offshore fisheries was over 10,000 metric ton in 1972, but thereafter gradually decreased and has been less than 2,000 metric ton for the last ten years. In response to this decrease of harvested resource, stock management plans are required. Although there are some studies on the migratory and distributional patterns of adults,[1-4] feeding habits[5] and the distribution of planktonic larvae[6] in the coastal waters of Hokkaido, there has been no accurate aging study, which is the basis for fisheries management decisions.

The objective of the present study was to validate the aging method using otoliths of pointhead flounder from Ishikari Bay, one of the major fishing grounds for this species.

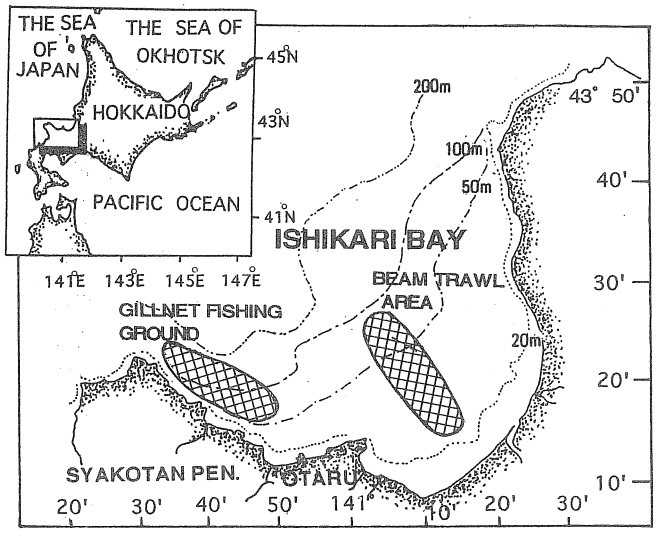

Fig. 1. Map showing sampling area and contours of depth (m) in Ishikari Bay, Hokkaido.

Materials and Methods

The samples used in this study were caught with commercial bottom gill nets in Ishikari Bay, Hokkaido (Fig. 1) from March to December, 1992 (Table 1). Fish were also obtained with an experimental beam trawl net (codend mesh aperture, 2.5 mm) aboard the R/V Oyashio-maru of Hokkaido Fisheries Experimental Station in Ishikari Bay from June 1991 to July, 1993 (Table 1). All specimens collected were sexed, measured to the nearest millimeter in standard length (SL), and weighed to the nearest 0.1 g. The largest otoliths, the saggitae, were removed and preserved in 70% ethanol for later analysis.

The otoliths of 0-age flounder could be read without polishing. However, annuli on the otoliths of 1-year-old and older flounder were not clear without polishing. Prior to processing, the otoliths were held in distilled water for 24 hours. The convex side of the otolith was polished with fine sandpaper (#600-#1,000 grit) until the focus became clearly visible. The otoliths were placed on glass slides and immersed in salad oil. Counts of annulus and otolith measurements were made under a binocular microscope at $\times 10$ magnification using reflected light.

There is a remarkable difference in the position of the fo-

Table 1 List of samples analyzed in this study

Sampling date	Sampling gear	Number of individuals					
		Male		Female		total	
24–26 Sep. 1991	beam trawl					11	
11 Nov. 1991	beam trawl					75	
24 Feb. 1992	beam trawl					107	
10 Mar. 1992	bottom gill net	48	(37)	89	(74)	137	(111)
28 Apr. 1992	bottom gill net	22	(22)	65	(54)	87	(76)
11 May 1992	bottom gill net	72	(58)	59	(49)	131	(107)
25 May 1992	beam trawl	43	(43)	43	(43)	86	(86)
24 Jun. 1992	bottom gill net	33	(23)	93	(83)	126	(106)
6 Jul. 1992	bottom gill net	34	(31)	75	(70)	109	(101)
14 Jul. 1992	beam trawl	72	(61)	37	(30)	109	(91)
12 Aug. 1992	bottom gill net	131	(41)	144	(65)	275	(106)
18 Sep. 1992	beam trawl	13	(11)	10	(7)	23	(18)
20 Oct. 1992	bottom gill net	5	(4)	87	(48)	92	(52)
21 Nov. 1992	beam trawl	46	(45)	29	(29)	75	(74)
10 Dec. 1992	bottom gill net	63	(41)	121	(80)	184	(121)
20 May 1993	beam trawl					77	
27 Jul. 1993	beam trawl					9	
Total		582	(417)	852	(632)	1,713	(1,049)

Numbers in parentheses indicates number of individuals used for otolith measurements.

cus[7–12] between the ocular side and the blind side of Pleuronectid fish otoliths. This fact also applied to the pointhead flounder used in the present study. Since otolith growth is faster on the axis of the ocular side of the body,[10] the ocular side otoliths can be used for reading, especially for the older fish. For this reason, they were used for the growth analysis of *Pleuronectes herzensteini*[10,13] and *H. dubius*.[12] Therefore, the ocular side otoliths were used for age determination and growth analysis in the present paper. Distances from the focus to the outer margin of each translucent zone (r_n) and the radius of the otolith (R) were measured to the nearest 0.05 mm along the longest axis from the focus to the anterior edge of the otolith (Fig. 2).

The regression method and Lee (Fraser-Lee)'s method[14] were used to estimate the back-calculated length. This regression method estimates the standard length (SL) at some previous time of annulus formation (n) by substituting the measured size of the otolith (r) at the time of annulus formation (n) into a fish length-otolith length regression derived from samples by sex,

$$SL_n = br_n + d \quad (1)$$

where b and d are the slope and intercept of the regression, respectively. Lee's procedure is described by the following equation:

$$SL_n = d + (SL_c - d) \times (r_n / R) \quad (2)$$

where SL_c is the standard length at capture.

Growth was assumed to conform to the von Bertalanffy growth function (VBGF), which was calculated using Ishizuka's computer program.[15]

Results

Period of Annulus Formation

Translucent zones and opaque zones were formed alternately on the otoliths. In order to clarify the period of annulus formation, monthly changes in the percentage occurrence of otoliths with a translucent edge (Fig. 3) were examined.

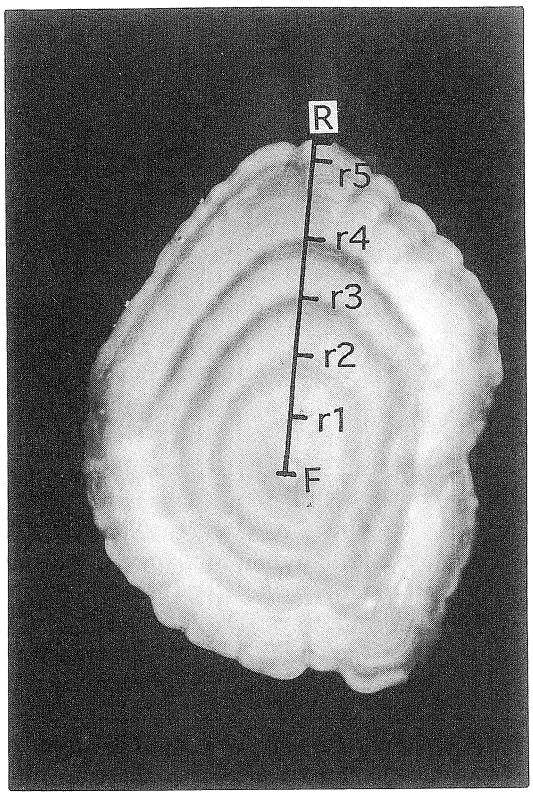

Fig. 2. Ocular side otolith of *H. pinetorum* with five annuli (male, 190 mm SL) collected on 11 May, 1992.
F, Focus; R, otolith radius; $r_1 - r_5$, annual ring radii.

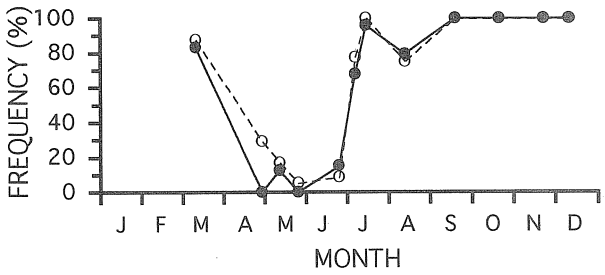

Fig. 3. Monthly changes in percentage of occurrences of otoliths with translucent edges for males (solid circles) and for females (open circles) *H. pinetorum*.
Samples were collected with commercial gillnet and an experimental beam trawl net from March to December 1992.

All fish otoliths had a translucent edge between September and December. The percentage occurrence of otoliths with a translucent edge began to decrease in March, and minimum values for both male (0%) and female (5.4%) were found in May, respectively. The values for both sexes increased in July. These results show that the outer translucent margin of the otoliths for both sexes is formed chiefly between March and April. No significant differences in percentage occurrence of translucent edges between sexes was observed except in April (Fisher's exact probability test, $p < 0.01$).

Growth Pattern of Otoliths

In order to elucidate the growth pattern of otoliths, monthly changes in the average marginal increments of otoliths (MI) were examined on the basis of 827 specimens possessing 1–10 ring marks:

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

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. Minimum values of marginal increment for both sexes were found in March (Fig. 4). Marginal increments then increased rapidly from May to August, reaching a maximum value between September and December.

Standard length-Otolith Radius Relationship and Back-Calculated Length

Plots of the relationships between otolith radius (R in mm) and standard length (SL in mm) are shown in Fig. 5. Least-square equations for both sexes are:

male: $SL=44.07R-0.39$ $(n=417, r^2=0.949)$ (3)
Female: $SL=46.42R-2.11$ $(n=632, r^2=0.941)$ (4)

The residuals and slope of the regression lines were significantly different between sexes (ANCOVA, residuals: $p<0.01$; slope; $p\ll0.01$). The mean radii of each successive otolith annulus at the time of annulus formation are shown in Table 2. Lee's phenomenon was found in r_1 and r_2 for both sexes but a clear Lee's or reverse-Lee's phenomenon was not found in r_3-r_9 for either sex. No significant differences between sexes were seen in the mean values of r_1-r_3, while the mean values of r_4-r_8 of females were larger than those of males (t-test, $p<0.01$). The mean standard lengths at the time of the n-th annulus formation (SL_n) were back-calculated from each mean radius by equations (3) and (4) and Lee's method (Table 3). The differences in standard lengths at the time of each annulus formation obtained by these two methods were similar.

There were significant differences (t-test, SL_2: $p<0.05$; others: $p<0.01$) in mean standard length at the time of annulus formation between sexes except for SL_1.

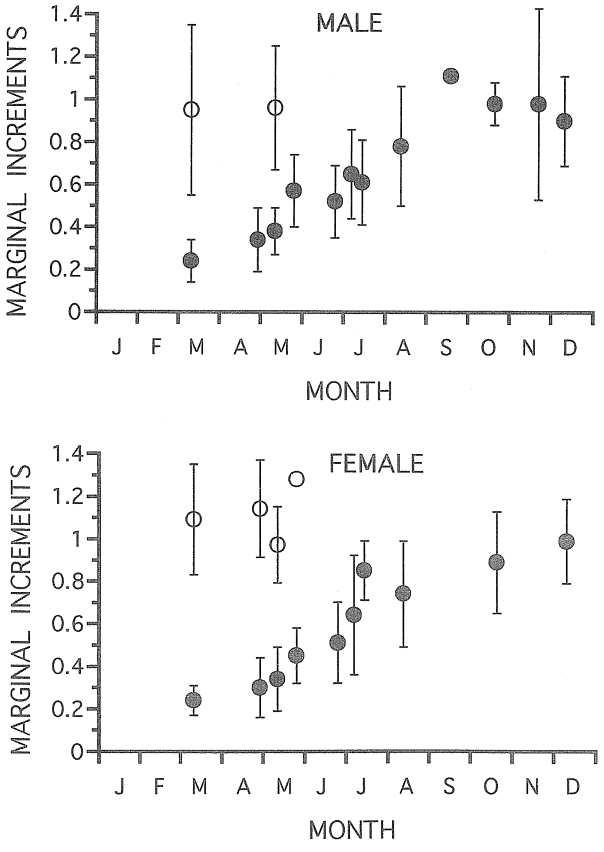

Fig. 4. Monthly changes in marginal increment of otoliths of *H. pinetorum* collected by commercial gillnet and experimental beam trawl net from March to December 1992.

Vertical bars indicate standard deviations. Open circles indicate the mean marginal increments of otoliths which do not have new opaque zones from March through May.

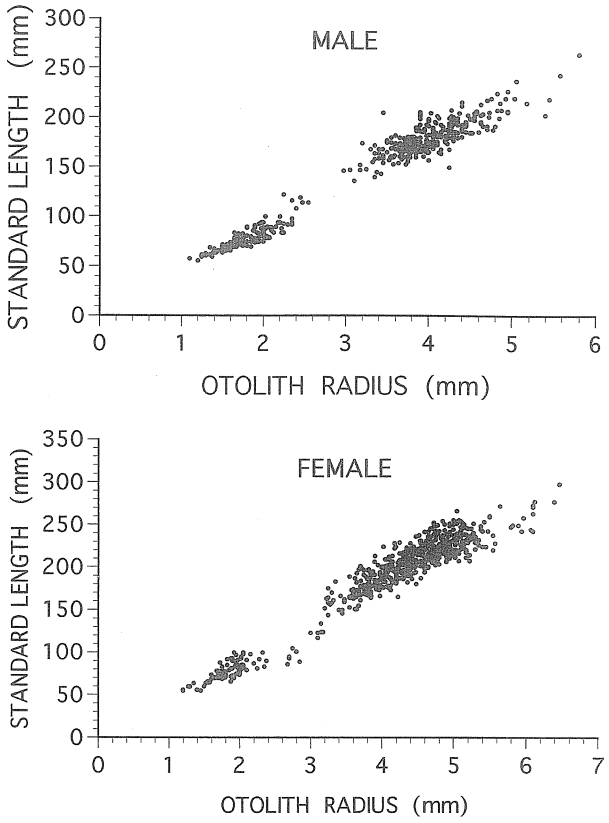

Fig. 5. Relationships between otolith radius and standard lengths of *H. pinetorum* for males and females.

Growth

The major spawning season of pointhead flounder is from June to July in Ishikari Bay.[*4] However, the annuli were formed chiefly between March and April, three or four months before the spawning season. Thus, VBGF curves were computed using back-calculated length and age (t−0.3) by Ishizuka's program.[15] The growth curves described by this program were as follows:
Regression method:

Male: $L_t=251.9(1-\exp^{-0.261(t-0.142)})$
Female: $L_t=315.1(1-\exp^{-0.219(t-0.200)})$

[*4] T. Tanaka, Y. Hinata, M. Yamamoto, T. Fukui, H. Kitahama, and K. Hayashi: Sohachi. Showa 36–40 nendo Report of Hokkaido coastal fisheries research and fisheries business economics experiments

Table 2 Mean ring radii (±SD) for each ring group of *H. pinetorum*

Ring groups	N	Ring radii (mm)									
		r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}
Male											
1	135	0.82±0.15									
2	18	0.87±0.16	2.03±0.29								
3	127	0.82±0.18	2.05±0.25	3.09±0.26							
4	64	0.73±0.15	1.72±0.27	2.73±0.34	3.60±0.36						
5	57	0.70±0.18	1.62±0.30	2.56±0.36	3.44±0.37	4.08±0.33					
6	7	0.71±0.13	1.72±0.12	2.82±0.24	3.63±0.20	4.19±0.19	4.56±0.23				
7	3	0.60±0.07	1.57±0.41	2.53±0.53	3.28±0.48	3.92±0.35	4.27±0.38	4.51±0.31			
8	3	0.70±0.07	1.63±0.20	2.56±0.23	3.18±0.20	3.66±0.09	4.09±0.07	4.42±0.11	4.69±0.13		
9	2	0.58±0.04	1.68±0.18	2.76±0.05	3.41±0.30	3.93±0.39	4.34±0.55	4.69±0.58	4.98±0.57	5.23±0.67	
10	1	0.45	1.03	2.88	3.53	3.90	4.30	4.60	4.95	5.10	5.35
weighted Mean	417	0.78±0.17	1.86±0.33	2.86±0.37	3.51±0.36	4.06±0.33	4.37±0.31	4.53±0.29	4.83±0.31	5.18±0.48	5.35
Female											
1	87	0.85±0.17									
2	23	0.89±0.17	2.15±0.28								
3	176	0.84±0.20	2.09±0.32	3.17±0.32							
4	170	0.75±0.16	1.83±0.30	2.85±0.37	3.88±0.40						
5	157	0.74±0.14	1.66±0.29	2.58±0.34	3.55±0.32	4.43±0.34					
6	10	0.75±0.19	1.69±0.30	2.76±0.42	3.57±0.52	4.37±0.53	5.01±0.54				
7	1	0.30	1.05	1.90	3.00	3.75	4.30	5.65			
8	1	0.50	1.03	1.83	2.73	3.40	3.88	4.78	5.08		
9	5	0.73±0.19	1.60±0.23	2.54±0.20	3.40±0.19	4.00±0.20	4.62±0.18	5.16±0.24	5.54±0.18	5.86±0.14	
10	2	0.78±0.18	1.95±0.46	2.98±0.32	3.79±0.58	4.35±0.53	4.78±0.46	5.06±0.51	5.39±0.41	5.76±0.30	5.99±0.27
weighted Mean	632	0.79±0.18	1.87±0.36	2.87±0.42	3.71±0.41	4.41±0.37	4.78±0.51	5.15±0.34	5.44±0.26	5.83±0.17	5.99±0.27

N: number of otoliths used.

Table 3 Mean back-calculated standard length (mm) at the time of each annulus formation by Regression method and Lee's method

	SL_1	SL_2	SL_3	SL_4	SL_5	SL_6	SL_7	SL_8	SL_9	SL_{10}
Regeression										
Male	34.1	81.6	125.8	154.4	178.5	192.3	199.2	212.3	228.0	235.4
Female	34.6	84.7	131.1	170.1	202.4	219.9	236.8	250.6	268.6	275.8
Lee										
Male	34.0	82.6	127.0	152.9	176.7	191.3	202.6	215.5	229.4	231.3
Female	34.7	85.7	132.4	171.6	204.9	224.9	243.0	258.0	275.0	286.8

Lee's method:

Male: $L_t = 251.6(1 - \exp^{-0.263(t-0.142)})$

Female: $L_t = 333.0(1 - \exp^{-0.204(t-0.186)})$

where L_t is the standard length (mm) at estimated age t (years).

Theoretical lengths for males estimated by the two back-calculated methods agreed very well but those for females were slightly different beyond 6–7 years (Fig. 6).

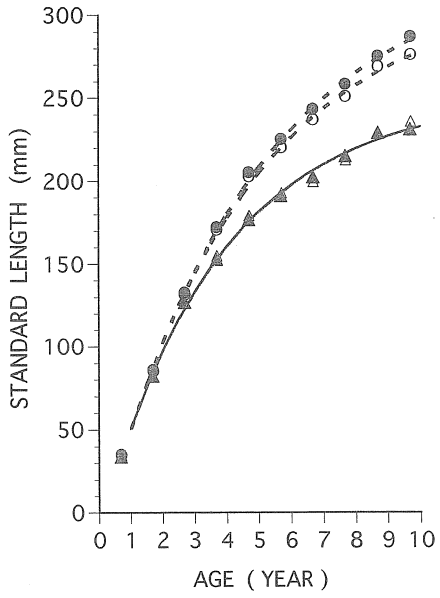

Fig. 6. von Bertalanffy growth curves for *H. pinetorum* for males (solid lines) and females (dashed lines).
The plots represent back-calculated standard length; open triangles: males by regression method; closed triangles: males by Lee's method; open circles: females by regression method; closed circles: females by Lee's method.

Growth of the 1991 Year Class

Post-settlement juveniles of the 1991 year class were not captured during the experimental beam trawl surveys in June and July 1991. The temporal growth patterns of the 1991 year class collected from September 1991 to July 1993 are shown in Fig. 7. Eleven juveniles of the 1991 year class ranging from 16 to 33.6 mm SL were collected by beam trawl in September 1991. In November 1991, the average standard length of the 1991 year class was 38.6 mm (SD 5.7 mm), which was almost the same size as back-calculated SL_1. The average standard length in February 1992

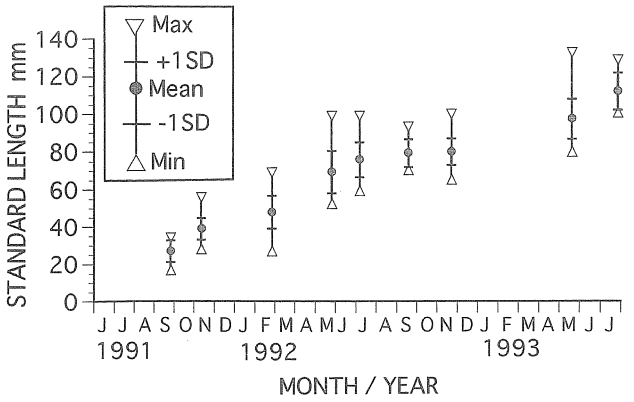

Fig. 7. Monthly change in standard length of the 1991 year class *H. pinetorum*.

Table 4 Mean standard length (mm) at each age of *H. pinetorum* calculated by von Bertalanffy Growth Function (Reg.: back-calculation by Regeression method; Lee: back-calculation by Lee's method) and those in the commercial landing in May 1992 (Observed: mean ±SD)

Age	Male			Female		
	Reg.*	Lee*	Observed*	Reg.*	Lee*	Observed*
3	132.5	132.8	160.6±7.2	144.3	145.5	170.7±8.1
4	160.0	160.3	168.7±5.3	177.9	180.1	193.9±11.5
5	181.1	181.4	184.7±12.3	204.8	208.3	220.7+13.9
6	197.4	197.6	200.8±17.3	226.5	231.4	

* Reg. and Lee: collected by Beam Trawl net.
Observed: collected by Gill nets4.

(47.1 mm) was slightly smaller than the theoretical standard length of 1-year-old fish. Growth rates from February to July were higher (c.a. 5.6 mm/month) than from July to November (c.a. 1.1 mm/month). The mean standard length in May 1993 was 97.0 mm and it was almost equal to the theoretical length of 2-year-old fish.

Discussion

All growth back-calculation procedures are based on the proportionality between fish length and some measure of otolith size.[14] Regression methods are commonly used for back-calculation.[10–13,16–19] Regression-based back-calculation assumes no deviation from the overall regression. However, the otolith radius varied in specimens of the same length. In order to remove the variation of ring radii, Mio[20] proposed that each radius be standardized by the following equation:

$$r_n' = (R'/R) \times r_n$$

where r_n' is the standardized ring radius at the time of n-th ring formation and R′ is an estimate derived from SL at capture of each specimen in the R-SL regression. In contrast, Lee's method[14] assumes that individual SL-R deviations are maintained proportionally throughout the back-calculation. Lee's formula has been proposed as the most suitable equation for deriving back-calculated fish lengths using scales.[14] In the present study, although small differences in mean back-calculated lengths were found between the two methods, back-calculated lengths were very similar at most estimated ages. Although it is impossible to estimate an individual growth trajectory by using the regression method, it may be appropriate to use the mean back-calculated length in this study.

For the 1991 year class, the observed mean length of the fish collected by the experimental beam trawl net in late February 1992 was similar to the theoretical length predicted by VBGF at 1-year-old, and that in middle May 1993 agreed very well with the theoretical length at 2-year-old. The mean observed lengths of the 3–6 year-old fish collected by commercial gill nets in May 1992 were larger than the theoretical lengths for both males and females. However, the difference between the observed and theoretical mean lengths became smaller with increasing age (Table 4). The majority of pointhead flounder in the commercial landings ranged from 190 to 220 mm SL in Ishikari Bay.*5 Therefore, it is clear that the difference between the observed and theoretical mean lengths is closely associated with the mesh selectivity of gill nets.

The asymptotic maximum standard lengths were estimated to be 251.9 mm (regression) and 251.6 mm (Lee) for males and 315.1 mm (regression) and 333.0 mm (Lee) for females, while the observed maximum lengths of males and females were 262 mm and 316 mm, respectively. Judging from the above results, it seems reasonable to suppose that the theoretical standard length indicates the approximate actual length at each age except for age 1. The time of opaque zone formation of 0-age fish appears to be earlier than that of other ages.

Previous studies on the growth of this species were also made in south-western Japan Sea[21–23] and Hachinohe, Pacific coast of northern Honshu Island.[9] In the Hachinohe region, the relationship between the time of annulus formation and the spawning season was not clear. However, the time of annulus formation was related to the spawning season in the southwestern Japan Sea,[21–23] so we compared the growth data from the southwestern Japan Sea[23] with our data. SL values of our data were converted to total length (TL) using the following equation:

$$TL = 1.19SL + 2.83 \quad (n = 927, r^2 = 0.996)$$

The parameters of VBGF of pointhead flounder from the southwestern Japan Sea were recalculated by Ishizuka's program from the age-length data presented elsewhere.[23] Pointhead flounder in the southwestern Japan Sea are larger than those in Ishikari Bay except for 1-year-old fish (Table 5). The difference of growth between the above two areas is caused mainly by differences in growth rates (mm/year) of 1- and 2-year-old groups (Table 5).

In the southwestern Japan Sea, large differences (50–60 mm) in mean body length of 1- and 2-year-old fish between the west fishery ground and the east fishery ground were found.[21,24] Watanabe[24] suggested that the differences might be caused by not only geographical differences in the food amount for 1- and 2-year-old fish and the fish density but also a distributional relation between flathead flounder and other fishes.

*5 O. Tominaga, Y. Watanabe and K. Domon: Sohachi. Heisei 4 nendo Annual Rep. Hokkaido Chuo Fish. Exp. Stan., 9–15 (1993).

Table 5 Mean total length calculated by von Beratlanffy Growth Function (VBGF) of *H. pinetorum* from South-western Japan Sea (SW Jap. Sea) and Ishikari Bay. Reg.

	Total Length calculated by VBFG mm						Growth rates (mm/year)					
	Male			Female			Male			Female		
	SW Jap.	Ishikari Bay		SW Jap.	Ishikari Bay		SW Jap.	Ishikari Bay		SW Jap.	Ishikari Bay	
	Sea	Reg.	Lee	Sea	Reg.	Lee	Sea	Reg.	Lee	Sea	Reg.	Lee
1	56.0	63.0	63.2	53.4	63.0	63.5						
2	135.5	118.1	118.4	141.0	124.9	125.4	79.5	55.1	55.2	87.6	61.9	61.9
3	187.5	160.5	160.9	205.1	174.5	176.0	52.0	42.4	42.5	64.1	49.6	50.6
4	221.4	193.2	193.6	252.2	214.5	217.1	33.9	32.7	32.7	47.1	40.1	41.2
5	243.6	218.3	218.7	286.7	246.5	250.7	22.2	25.1	25.1	34.5	32.0	33.6
6	258.1	237.7	238.0	312.0	272.4	278.2	14.5	19.4	19.4	25.3	25.8	27.5
7	267.6	252.7	252.8	330.6	293.1	300.4	9.5	15.0	15.0	18.6	20.7	22.3
8	273.7	264.2	264.3	344.1	309.7	318.7	6.1	11.4	11.4	13.5	16.7	18.2
9	277.8	273.0	273.1	354.1	323.1	333.5	4.1	8.8	8.8	10.0	13.3	14.9
10	280.4	279.7	279.7	361.4	333.8	345.7	2.6	6.7	6.6	7.3	10.7	12.2

Back-calculation by Regression method; Lee: back-calculation by Lee's method. Parameters of VBGF for *H. pinetorum* from south-western Japan Sea were recalculated as follows: $TL_t=285.4(1-\exp^{-0.426(t-0.487)})$ for male, $TL_t=381.5(1-\exp^{-0.310(t-0.514)})$ for female based on the data of Done.[23] Maximum age in the study was 6 years old (one specimen) for male and 7 years old (two specimens) for female.

The 1- and 2-year-old groups inhabit the shallow waters in Ishikari Bay while the main habitat of fish aged 3 years and older is in the deep area out beyond Ishikari Bay.[25] To confirm the cause of the geographical variation in growth of pointhead flounder, studies on fish biomass, distribution and feeding habits of 1- and 2-year-old fish combined with biological, physical and chemical environment factors are required.

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