

Evaluation of the Precision of Age Determination and Variability in Growth for Japanese Sardine (*Sardinops melanostictus*), Tsushima Warm Current Subpopulation

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Abstract

Accurate estimates of fish age and growth are essential for stock assessment and management. We evaluated the differences in aging precision of Japanese sardines (*Sardinops melanostictus*) from the Sea of Japan and East China Sea determined from the counts of annual rings on scales and otolith observation by two readers. Changes in growth parameters between 2011 and 2018 year-classes were also evaluated. Although the aging precision between readers was relatively precise for both scales and otoliths, otoliths may be more useful for determining the age of this population because of the low discrepancy of the age counts between readers. The average standard length of Japanese sardines aged 2-3 years was relatively stable for 2011-2017 year-classes but declined sharply after 2018 year-class, along with increased stock biomass. Recent (2018 year-class) estimated theoretical asymptotic length also declined compared with those of early 2010s year-classes. Changes in the mean standard length at an age of 2-3 years and theoretical asymptotic length of Japanese sardines in the Sea of Japan and East China Sea may be a density-dependent response to increased stock abundance.

Discipline: Fisheries

Additional key words: aging accuracy, aging method, density dependence, otolith, scale

Introduction

Japanese sardines (*Sardinops melanostictus*) are widely present in the waters around Japan and are one of the most important species for the Japanese fishery. Two subpopulations occur: northwestern Pacific (Pacific subpopulation) and Sea of Japan and East China Sea (Tsushima Warm Current subpopulation; the latter hereinafter referred to as the TWC subpopulation). The TWC subpopulation has a large fluctuation of stock biomass (Nakai 1962, Hiyama et al. 1995, Ohshimo et al. 2009, Muko et al. 2022), and this may be related to the changing climate (Hiyama et al. 1995, Ohshimo et al. 2009) and/or differences in the timing of phytoplankton blooms (Kodama et al. 2018). For both subpopulations, the total catch increased from the early 1970s, peaked in the late 1980s, and declined sharply in the early 1990s (Ohshimo et al. 2009). Although both the stock biomass and spawning stock biomass of the TWC subpopulation

remained low until 2010, catch then increased (Fig. 1A) and the stock has both recovered and increased sharply since 2020 (the stock biomass and spawning stock biomass of the TWC subpopulation in 2019-2021 were 0.198, 0.398, and 0.541 million tons, and 0.082, 0.143, and 0.274 million tons, respectively) (Fig. 1 B, C; Muko et al. 2022). This recent trend may be similar to that in the early 1970s based on estimates reported in a most recent stock assessment (Muko et al. 2022).

The TWC subpopulation is widely distributed throughout the Sea of Japan and East China Sea (Muko et al. 2018), but this distribution expands when stock levels are high and contracts to coastal waters when stock levels are low (Hiyama 1998). Biological characteristics, including growth and reproduction of this species, also change with stock biomass (e.g., Hiyama et al. 1995, Yoneda et al. 2013). For instance, the body length of older individuals is smaller at higher stock sizes (Hiyama et al. 1995, Morimoto 2003, Kim et al. 2006), with the negative

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correlation between body length and stock biomass possibly density-dependent (Hiyama et al. 1995, Ohshimo et al. 2009). In addition to these, Ohshimo et al. (2009) reported that there is a positive relationship between the body length at age 3 of the TWC subpopulation and the wet weight of zooplankton in the offshore area of the Sea of Japan. Furuichi et al. (2020) suggested that climatic factors (i.e., sea surface temperature) cause shifts and expansion or contraction of Japanese sardine spawning grounds and shifts in spawning timing. As mentioned above, the ecological and biological characteristics of Japanese sardines may be affected by population abundance and environmental change.

The current methodology of stock assessment of the TWC subpopulation is an age-structured model (Muko et al. 2022). Thus, accurate age determination of caught fish is essential to obtain reliable age composition of the

catch for the stock assessment. Scales have been commonly used to determine the age of Japanese sardines (Hiyama et al. 1995, Ohshimo et al. 2009), but these can easily detach from the body and can be replaced (i.e., regenerated scale). Therefore, other hard tissues (i.e., otoliths) are generally used to determine the age of other sardines (Fletcher & Blight 1996, Yaremko 1996, van Beveren et al. 2014). Although procedures are established to observe the age rings in the otoliths of the Pacific subpopulation (Kamimura et al. 2022), the accuracy of using scales and otoliths to determine age is unknown. Therefore, our objectives are the following: 1) to evaluate the precision of age determination using scales and otoliths and 2) to evaluate variability in growth for the TWC subpopulation of Japanese sardines in recent decades.

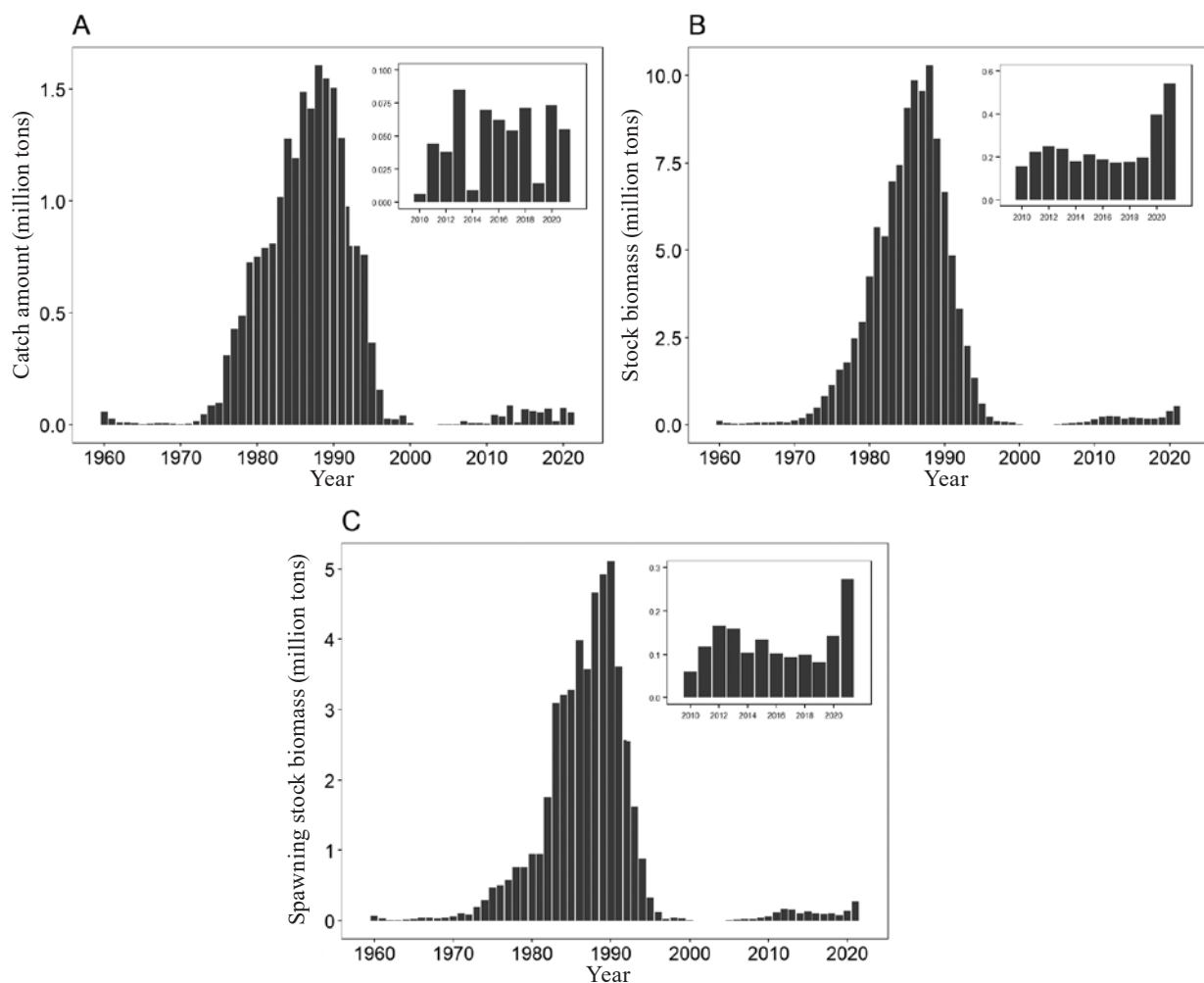


Fig. 1. Yearly changes in A catch amount, B stock biomass, and C spawning stock biomass of Japanese sardines in the Sea of Japan and East China Sea, based on the most recent stock assessment (Changed from Muko et al. 2022, with permission)

Materials and methods

1. Age determination

Japanese sardines were captured by Japanese research vessels (surface–midwater trawl nets) and by commercial vessels (purse seine and set nets) operating in the Sea of Japan and the East China Sea (Fig. 2). In the laboratory, standard length (SL) was measured to the nearest millimeter. The scales of a fish were taken from the lateral side of the body, washed in fresh water, and placed between two glass slides (Nakai 1962). Scale samples ($n = 12,870$) collected between 1964 and 2022 were available from the archived collections of the Fisheries Resources Institute (Nagasaki, Japan). Of these, we used the age and SL data for 6,654 individuals caught from 2011 to 2022 to elucidate variability in growth for this relatively recent period of time. After the exception of the regenerated scales, the age rings (translucent and opaque zones) of the scales were counted without prior knowledge of fish SL or counts of another reader using the RATOC otolith measurement system (ARP/W, RATOC System Engineering).

Sagittal otoliths of individuals caught between 2011 and 2022 were removed from the inner ear, washed in fresh water, and air-dried. The otolith surface was observed while immersed in 99% ethanol in a glass petri dish against a black background in accordance with the procedure of Kamimura et al. (2022). The age rings on the otolith surface were counted with the same procedure as that of the scales using the RATOC otolith measurement system.

To evaluate the aging accuracy of both scales and otoliths, two persons read random subsamples ($n = 192$, SL: 99 mm - 238 mm) using the RATOC otolith measurement system. The aging precision of the counts made by different readers for both scales and otoliths was evaluated based on percent agreement (PA), average percent error (APE; Beamish & Fournier 1981), and mean coefficient of variation (CV; Chang 1982). To determine whether differences in counts between readers and between scales and otoliths were systematic or were due to a random error, aging bias was investigated using age-bias plots (Campana et al. 1995).

To validate the periodicity of age ring formation, the

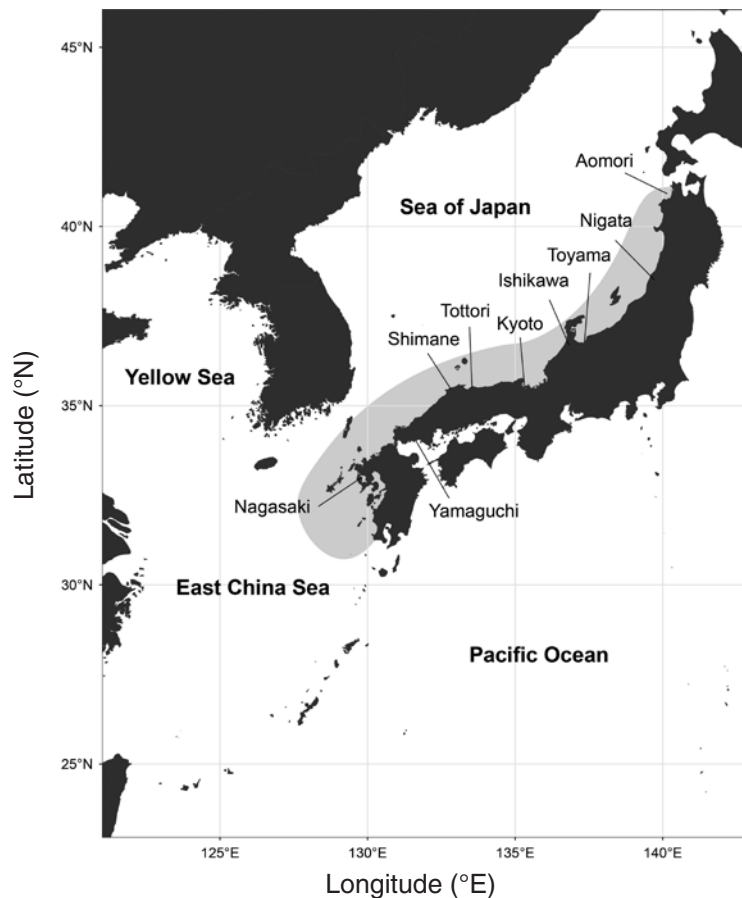


Fig. 2. Map of the research and fishing locations for Japanese sardines in the Sea of Japan and East China Sea

most peripheral zone on each otolith was classified as either translucent or opaque. We then analyzed the monthly changes in the frequency of each zone on the otolith margin throughout the year. Because the main spawning period in the TWC subpopulation extends from February to April (Ohshimo et al. 1997), we assumed the hatch date of all individuals to be 1 March. On this basis, we calculated the age of each individual.

2. Growth estimation

We averaged SL at age 2, 3, and >3 years using data from 2010 to 2022 to determine if a negative correlation between SL and stock biomass exists (Hiyama et al. 1995, Ohshimo et al. 2009).

The von Bertalanffy growth function (VBGF) was fitted to observe the length at age data for 2011 to 2018 year-classes using a nonlinear least-squares regression

as follows:

$$L_t = L_\infty [1 - \exp\{-K(t - t_0)\}],$$

where L_t is the predicted length at age t (in years), L_∞ is the theoretical asymptotic length, K is a growth coefficient (per year), and t_0 is the hypothetical age at zero length. VBGF parameters were estimated using the “FSA” package (Ogle et al. 2020) in R software (version. 4.1.2, R Core Team 2021). The Akaike’s information criterion (AIC) was also calculated for each year-class and pooled year-classes to determine whether parameters were year-class dependent.

Results

We used the age and SL data of 6,654 individuals

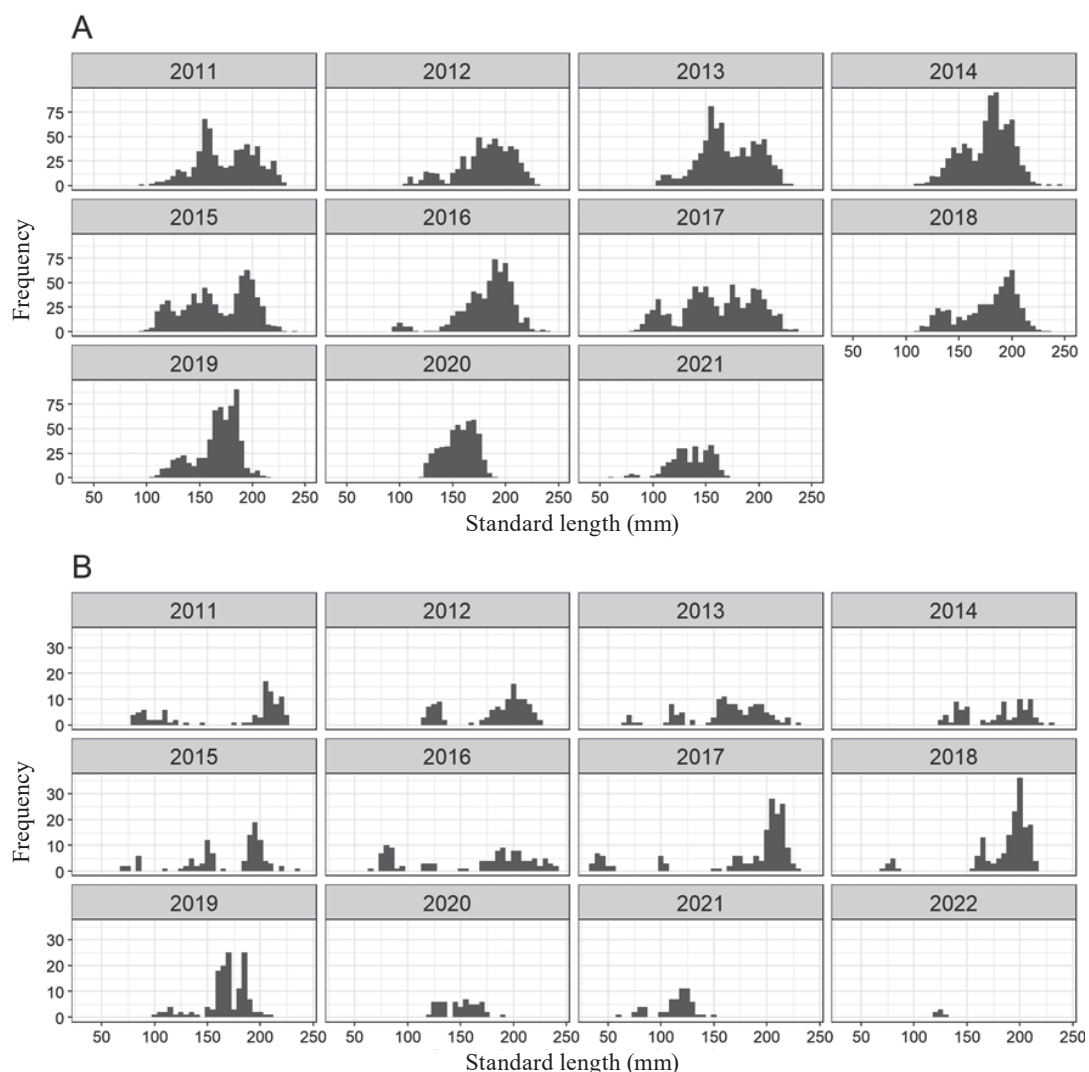


Fig. 3. Length–frequency distribution of Japanese sardines determined from **A** fish scales and **B** otoliths by year-class

(SL: 61 mm - 243 mm; Fig. 3A) for scale-based age determination and 1,214 individuals (SL: 36 mm - 240 mm; Fig. 3B) for otolith-based age determination. The number of age rings ranged 0 - 6 for both scales and otoliths, and the estimated age was 0.0 - 6.2 years for scales and 0.1 - 6.3 years for otoliths. Between-reader agreement in age ring counts for both scales and otoliths was relatively high (Fig. 4 A,B), but errors associated with age estimates of

individuals >3 years were greater than those associated with ages for younger individuals. Between-reader evaluations of precision using scales and otoliths were PA (72.4% and 78.7%), APE (5.80% and 4.62%), and CV (8.21% and 6.53%), respectively (Table 1). Although there was no systematic error in reader bias for age counts of individuals aged <4 years based on either scales or otoliths, differences in age counts of older individuals

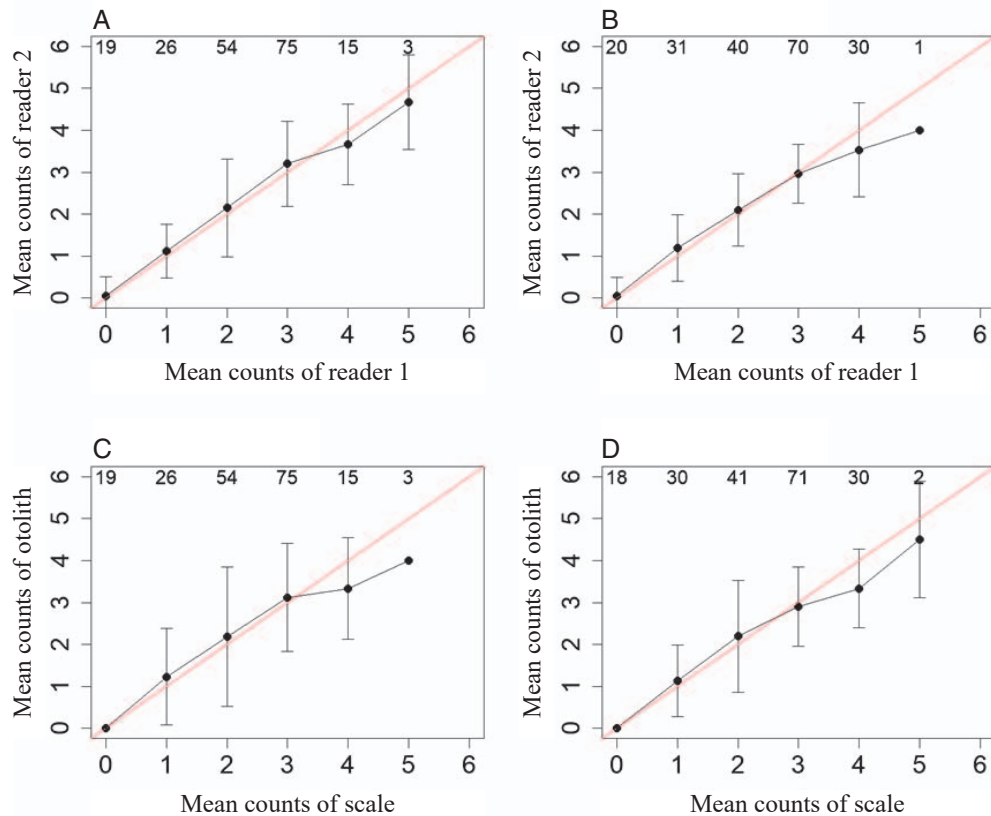


Fig. 4. Age-bias plots of pairwise comparisons of age rings between readers for A scales and B otoliths, and between scales and otoliths for C reader 1 and D reader 2

Error bars represent 95% confidence intervals. The diagonal line indicates 1:1 equivalence. Numbers at the top of the graph denote sample sizes for each count.

Table 1. Summary of aging precision between readers and between aging materials

Year-class	PA ^a	APE ^b	CV ^c
Between reader (scale)	72.4	5.80	8.21
Between reader (otolith)	78.7	4.62	6.53
Between material (Reader 1)	58.3	9.34	13.21
Between material (Reader 2)	69.8	6.34	9.10

^a PA, percent agreement

^b APE, average percent error

^c CV, mean coefficient of variation

were observed (Fig. 4 C,D).

We observed a marked difference in the proportion of translucent or opaque zone on the otolith margin throughout the year (Fig. 5). The proportion of opaque zones increased from boreal spring to summer, peaked in August, and was lowest in winter. By contrast, translucent zones were prevalent in winter and least common in summer.

The mean SL at age 2 calculated from both scales and otoliths declined sharply from 2019 (SL: scale, 178.1 mm; otolith, 175.1 mm) to 2020 (SL: scale, 171.3 mm; otolith, 169.9 mm) year-classes (Fig. 6). A decline in mean SL also occurred at age 3 from 2018 (SL: scale, 193.7 mm; otolith, 199.6 mm) to 2019 (SL: scale, 184.4 mm; otolith, 191.4 mm) year-classes. Although it is difficult to determine because of large variability, the mean SL at age >3 appears to have declined since 2017 year-class. Overall, the mean SL for the TWC subpopulation of Japanese sardines has been

decreasing since 2021.

VBGF parameters were estimated for 2011–2018 year-classes from 5,134 individuals using scales (Table 2) and 955 individuals using otoliths (Table 3). For scale- and otolith-based age determination, the sum of the AIC value for each year-class from 2011 to 2018 (scale, 42,295.3; otolith, 7,329.3) was lower than the value for all-year-classes pooled (scale, 43,317.5; otolith, 7,920.7), which suggests that parameters were year-class dependent. The VBGF parameters estimated from scale and otolith data showed roughly similar growth trajectories. For scale-based growth parameters, L_{∞} ranged from 202.8 to 237.9 mm, K ranged from 0.36 to 0.85, and t_0 ranged from -0.73 to -2.35 . For otolith, L_{∞} ranged from 200.5 to 234.1 mm, K ranged from 0.39 to 1.41, and t_0 ranged from -0.05 to -1.87 . Overall, L_{∞} in 2018 year-class (scale, 202.8 mm; otolith, 200.5 mm) was smaller than that in 2011 year-class (scale, 220.7 mm; otolith, 229.6 mm).

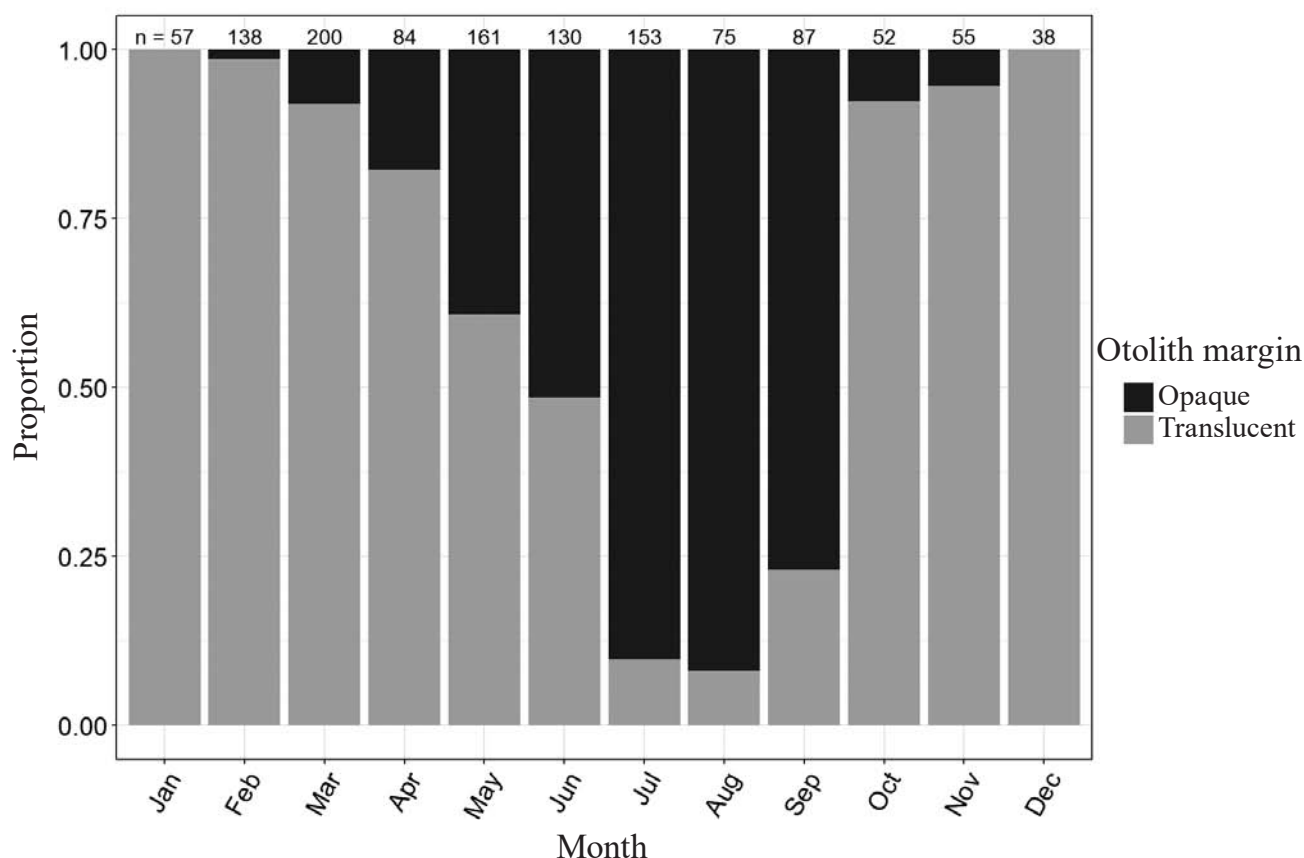


Fig. 5. Monthly change in frequency of occurrence of opaque and translucent bands on otolith margins of Japanese sardines from the Sea of Japan and East China Sea
Numbers above bars represent monthly sample size.

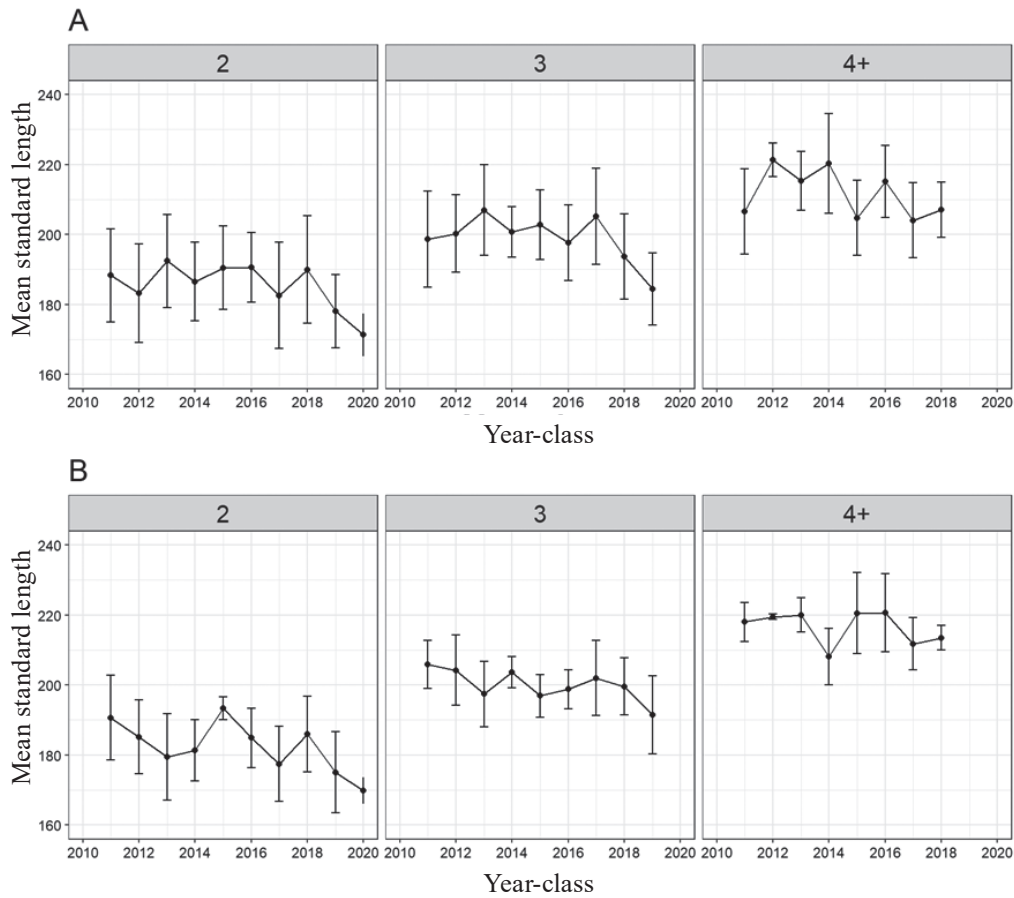


Fig. 6. Fluctuations in the mean standard length of Japanese sardines from the Sea of Japan and East China Sea determined using A scales and B otoliths at ages 2, 3, and 4+ (plus group) for 2011-2022 year-classes
Error bars represent standard deviations.

Table 2. Number of individuals (*n*), standard length (SL), age (*t*) range, and von Bertalanffy growth parameters with 95% confidence intervals (95% CI) by year-class estimated from scales of Japanese sardines from the Sea of Japan and East China Sea

Year-class	<i>n</i>	SL (mm)	<i>t</i> (year)	L_{∞} (95% CI)	K (95% CI)	t_0 (95% CI)	AIC ^a
2011	550	97-231	0.3-5.2	220.7 (211.7, 235.8)	0.40 (0.28, 0.54)	-2.35 (-3.16, -1.77)	4,412.1
2012	548	105-228	0.0-4.3	236.8 (221.6, 269.6)	0.36 (0.23, 0.48)	-1.89 (-2.64, -1.39)	4,573.8
2013	745	105-230	0.0-4.9	231.6 (222.5, 243.7)	0.47 (0.38, 0.56)	-1.37 (-1.65, -1.13)	5,998.8
2014	751	111-243	0.4-5.8	220.5 (209.8, 239.1)	0.44 (0.29, 0.60)	-1.99 (-2.82, -1.43)	6,235.6
2015	700	96-240	0.0-6.2	237.9 (225.5, 257.1)	0.37 (0.28, 0.45)	-1.80 (-2.21, -1.51)	5,850.9
2016	606	95-238	0.0-5.6	208.1 (204.6, 212.2)	0.73 (0.64, 0.84)	-0.89 (-1.10, -0.71)	4,908.5
2017	698	82-236	0.3-4.6	222.2 (213.5, 234.3)	0.53 (0.43, 0.64)	-1.09 (-1.34, -0.88)	5,935.5
2018	536	111-233	0.0-4.1	202.8 (198.8, 207.9)	0.85 (0.70, 1.01)	-0.73 (-0.97, -0.55)	4,380.1
All	5,134	82-243	0.0-6.2	214.6 (211.9, 217.6)	0.59 (0.54, 0.63)	-1.18 (-1.28, -1.09)	43,317.5

^a AIC, Akaike's information criterion

Table 3. Number of individuals (n), standard length (SL), age (t) range, and von Bertalanffy growth parameters with 95% confidence intervals (95% CI) by year-class estimated from otoliths of Japanese sardines from the Sea of Japan and East China Sea

Year-class	n	SL (mm)	t (year)	L_{∞} (95% CI)	K (95% CI)	t_0 (95% CI)	AIC ^a
2011	95	79-226	0.1-4.4	229.6 (220.7, 243.7)	0.56 (0.43, 0.72)	-0.71 (-0.94, -0.53)	715.9
2012	115	113-225	0.5-6.3	210.8 (205.7, 218.4)	0.94 (0.72, 1.19)	-0.47 (-0.73, -0.28)	858.2
2013	110	107-228	0.3-5.3	210.1 (199.5, 227.1)	0.54 (0.39, 0.72)	-1.20 (-1.71, -0.86)	873.7
2014	83	125-232	0.5-5.1	230.6 (214.5, 277.6)	0.39 (0.20, 0.56)	-1.87 (-3.08, -1.22)	633.1
2015	103	69-236	0.3-5.3	234.1 (217.1, 267.6)	0.47 (0.32, 0.62)	-0.72 (-1.09, -0.48)	854.3
2016	115	66-240	0.3-5.8	225.8 (218.7, 225.5)	0.68 (0.60, 0.81)	-0.34 (-0.47, -0.23)	921.3
2017	166	36-232	0.2-4.9	210.8 (207.4, 214.6)	1.07 (0.92, 1.23)	-0.03 (-0.09, -0.03)	1,326.2
2018	161	72-216	0.3-4.2	200.5 (198.7, 202.4)	1.41 (1.27, 1.55)	-0.05 (-0.11, -0.01)	1,146.4
All	955	36-240	0.1-6.3	211.6 (209.3, 214.1)	0.84 (0.77, 0.90)	-0.37 (-0.43, -0.32)	7,920.7

^a AIC, Akaike's information criterion

Discussion

Aging precision was generally similar using scales or otoliths, but that of otolith was lower than the reference APE and CV values (APE, 5.5%; CV, 7.6%; Campana 2001) obtained using a variety of aging structures (e.g., scales, otoliths, and vertebrae), indicating the greater reproducibility of using otoliths to determine age. A summary of 117 published precision values for several species (Campana 2001) suggests that the mean annual otolith ages tend to be slightly more precise than those based on other structures (e.g., scales and vertebrae). Although age counts for older individuals (aged >3 years) were slightly different between scales and otoliths (Fig. 4), scale- and otolith-based growth parameters had similar growth trajectories. This indicates that the differences between the aging structures (i.e., scales and otoliths) did not affect age determination of this species. However, scales can be somewhat unreliable because they easily detach from the body and can be replaced, and otoliths would be preferable to use for Japanese sardine age determination, in terms of usefulness. In conclusion, otoliths are a viable alternative to scales for determining the age of the TWC subpopulation of Japanese sardines.

Our results showed that the proportion of opaque zones on the outer margin of otoliths increased from boreal spring to summer (peaked in August), and translucent zones were prevalent in winter and least common in summer. The proportion of opaque zones in the Pacific subpopulation of Japanese sardines was higher from May to September in all age classes (Kamimura et al. 2022). Therefore, we assert that age ring formation in the TWC subpopulation occurs annually, regardless of geographic area of sampling used to estimate ages.

We report that the average SL at age 2 and 3 years remained relatively stable for 2011 to 2017 year-classes, but as stock biomass increased, this declined sharply for year classes after 2018 (Fig. 6). This relationship between decreased SL and increased stock biomass has been previously reported for both TWC and Pacific subpopulations (Hiyama et al. 1995, Morimoto 2003, Ohshimo et al. 2009, Kamimura et al. 2022). The theoretical asymptotic length estimates of the TWC subpopulation also declined in 2018 year-class compared with those from the early 2010s (Tables 2, 3). Our growth trajectory estimates based on scales generally lie within ranges reported by Ohshimo et al. (2009) for growth in 1980-1987 year-classes ($L_{\infty} = 218.7$) when stock biomass was high and 1973-1979 and 1988-2005 year-classes ($L_{\infty} = 250.2$) when stock biomass was low (Fig. 7B). However, the estimated L_{∞} for 2018 year-class was smaller than that reported for 1980-1987 year-classes.

The mean SL at age 2-4 of the Pacific subpopulation declined sharply since 2015 or 2016 year-class as stock abundance increased, and L_{∞} estimates also declined since 2015 year-class (Kamimura et al. 2022). By contrast, the mean SL and theoretical asymptotic length estimates of the TWC subpopulation might decline after 2018 year-class (Tables 2, 3; Fig. 6). The lag in timing of the recent growth decline between the Pacific subpopulation and TWC subpopulation could be due to the time difference in the period of stock increase between the two subpopulations.

Our results (Figs. 6, 7) suggest that growth changes in the TWC subpopulation may be a density-dependent response to increased stock abundance, as reported for the Pacific subpopulation (Kamimura et al. 2022). However, the stock abundance of the TWC subpopulation between 2010 and 2021 remained relatively low (0.156 - 0.541

million tons) compared with values between 1980 and 1989 (4.252 - 10.299 million tons) (Muko et al. 2022). In the 1980s and 1990s, the average SL of the TWC subpopulation decreased from 1982 to 1983 (Hiyama et al. 1995, Ohshimo et al. 2009), when stock abundances were 5.400 and 6.984 million tons, respectively. If the stock abundance of the TWC subpopulation continues to increase in the future, the growth of this subpopulation might change more drastically. Furthermore, changes in body size could be not only due to density-dependent effects but also due to other several factors, including food availability and the ocean environment (e.g., Hiyama et al. 1995, Ohshimo et al. 2009). Therefore, continuous monitoring of growth patterns in this subpopulation and examining the relationship between growth and

environmental conditions (i.e., food availability and water temperature) are required for informed future stock assessment and management.

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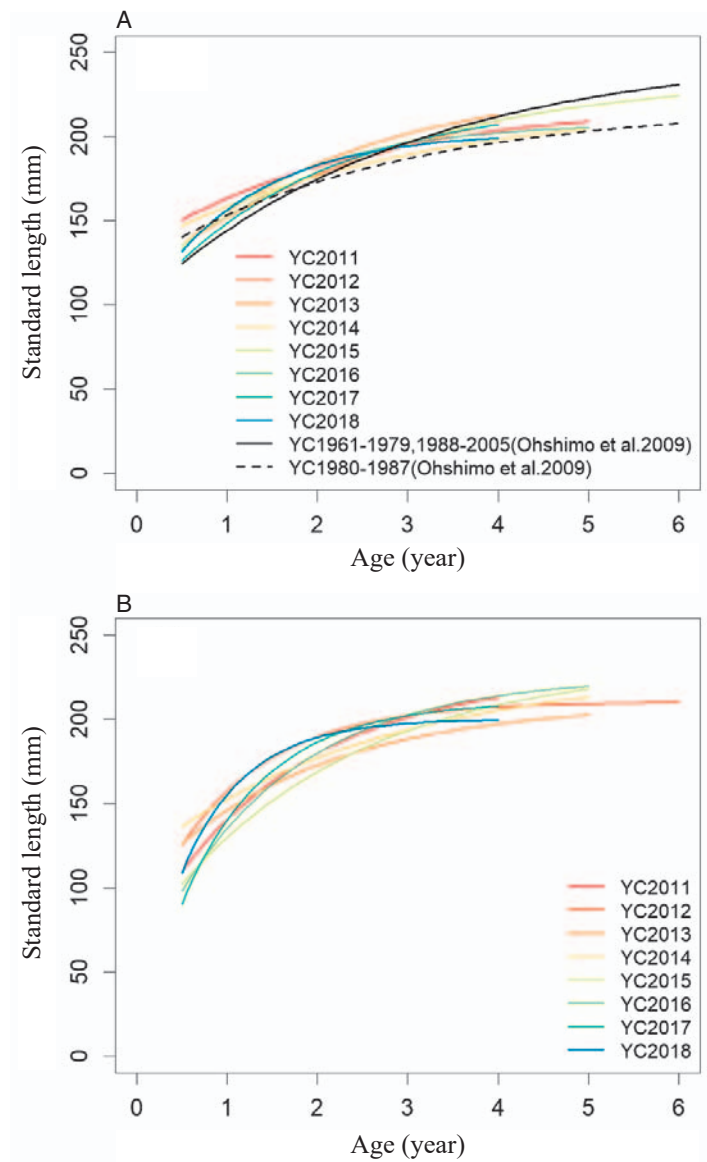


Fig. 7. Comparison of von Bertalanffy growth curves of Japanese sardines from the Sea of Japan and East China Sea estimated from scales A and otoliths B for 2011-2018 year-classes (YC)

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