

Age, Growth and Stock Status of Robust Tongue Sole *Cynoglossus robustus* Günther, 1873 in Japan Determined by a New Otolith Observation Technique

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Abstract

An ageing methodology was developed and used to examine age composition of robust tongue sole *Cynoglossus robustus* Günther, 1873 stocks inhabiting the Seto Inland Sea, Japan. Our sectioning and etching methods were powerful tools for identifying annual checks in otoliths, which were confirmed by scanning electron microscope observation of the otolith fine structure. These checks formed during the spawning period were also seen in immature fish. Using this new ageing methods, the age-length relationships and parameters for the von Bertalanffy growth function of male and female fish were determined to be $L_{\infty} = 324$ mm and 351 mm and $k = 1.16$ year⁻¹ and 1.05 year⁻¹, respectively. The age composition of the populations studied and of the landings of the bottom trawl fisheries showed the percent spawning potential ratio (%SPR) to be approximately 30%. Thus, fast growth of this species would maintain the population, even under fishing pressure.

Introduction

Year-class strength and age composition are the fundamental data used to assess fisheries stocks. Accurate and precise age information is necessary for such analyses. Surface observation of otoliths is widely used to determine age of teleost species. Sectioning methods (e.g., staining, burning, and UV-observation of burnt otoliths) also are commonly used ageing techniques. In general, it is fairly easy to use such methods to determine the age of flatfishes. However, only a few otolithometric studies of tongue fishes (i.e., the flatfish family known as Cynoglossidae) exist. Booth and Walmsley-Hart (2000) reported life history characteristics of the red spotted tongue sole

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Cynoglossus zanzibarensis Norman, 1939 based on growth traits observed in otolith sections. Kusakabe (2011) also utilised otolith sections to study age and growth of the robust tongue sole *Cynoglossus robustus* G nther, 1873.

The robust tongue sole is distributed in the western Pacific, along southern Japan, and in the South China Sea. It inhabits coastal sandy and muddy bottoms at 20–115 m depth. In Japan, tongue sole are commonly found in coastal sea areas and are widely consumed as food. In the present study, an otolithometric ageing method was developed and used to examine age and growth, age of maturation, and other life history traits of robust tongue sole stocks inhabiting the Seto Inland Sea, Japan. Using this new technique, we detected an annual structure in the sectioned otoliths. Based on biological characteristics and the population status presented herein, the percent spawning potential ratio (%SPR) of the population was calculated using a simple method based on the age composition (Katayama et al. 2010). The results were then used to evaluate fishing pressure on the population.

Materials and Methods

Sampling and measurements

Fish samples were collected using bottom trawls (2.0 cm mesh in cod end) in the central part of Seto Inland Sea, Japan (Fig. 1) in 1999-2000 and 2008. Monthly samples of 10-20 and around 50 specimens were randomly collected from August 1999 to June 2000 and from July 2000 to March 2001, respectively. In August 2008, additional 28 specimens were sampled to compensate the shortage of small sized fish in previous samplings. Approximately 100 fish per month were sampled randomly for most months. The total length (TL), body weight (BW), and gonad weight (GW) were measured for each fish to the nearest 1 mm and 0.1 g, respectively. Sexes were identified by macroscopic examination of the gonads. Maturation condition was determined using the following gonadosomatic index (GSI):

$$\text{GSI} = \text{GW (g)} * 100 / \text{BW (g)}$$

Otolith treatment

Sagittal otoliths were removed from each fish, cleaned, dried, and stored in natural condition until further observation. Otoliths were mounted and embedded in polyester resin and cut into 0.3 mm transverse sections using a diamond saw (Leica sp1600; Leica Microsystems GmbH, Wetzlar,

Germany). Sections were mounted on glass slides and their surfaces were ground to sequentially finer grades using carborundum paper. Next, they were etched with 0.2 N HCl for 40–60 seconds and examined using a binocular microscope under transmitted light. The range of magnification was 40x or 100x. To observe the fine microstructure of the annuli, a scanning electron microscope (SEM, Miniscope TM-1000, Hitachi High-Technologies Corporation, Tokyo, Japan) was used. The sectioned and etched otoliths observed under the microscope were then observed with SEM without special preparations.

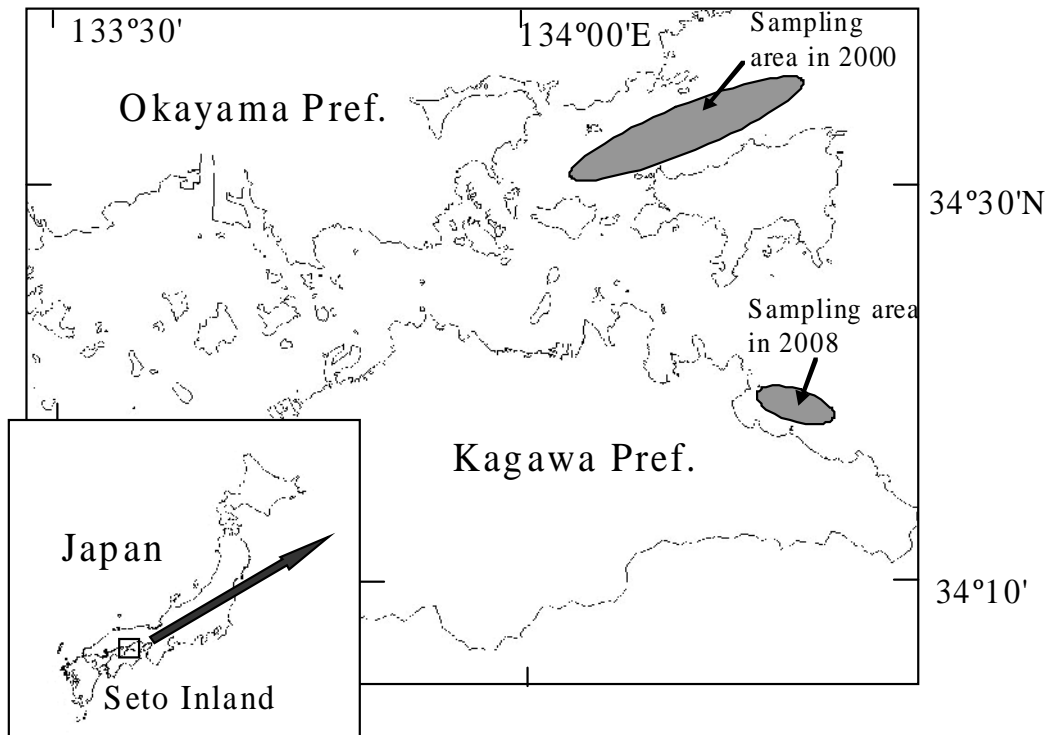


Fig 1. Map showing sampling areas in 2000 and 2008 (shaded area) of robust tongue sole investigated in this study.

Data analyses

Microsoft Excel's solver least squares cosine spectrum analysis was used to fit von Bertalanffy growth curves to the individual TLs of females and males at the age (year) estimated by observing otoliths. Assuming a birth date of June 1 (based on previously collected data), age of each individual was determined by observing the etched otolith section. Sample size of young-of-the-year was so small that t_0 (the theoretical age at zero length) of the growth curve was too large; therefore, the biological intercept method was used. This method requires only information about body length and the age relationship to calculate the intercept (i.e., total length of 1.8 mm at hatching day)

(Fujita et al. 1986). Thus, with this method all growth curves pass through the origin of coordinates with $t = 0$ and $TL = 1.8$. Analysis of covariance was conducted to examine whether the growth curves differed significantly between sexes.

Percent spawning potential ratio

To evaluate fishing pressure, Katayama et al. (2010) proposed that %SPR can be estimated easily using age composition data even if samples are not continuous. Natural mortality for all three species in their study was set at $2.5 \text{longevity}^{-1}$ based on Tanaka (1960), assuming that the longevity was the maximum age recorded for each species in the study. The number of fish at ages recruited to the fishery directly from the observed age composition, and the potential relative number of fish at ages using only natural mortality and recruitment, were derived. Spawning stock biomass was calculated using sex ratios, female maturity rates, and female weight. Sex ratio in the sample was examined by the z -test for a proportion.

Results

Otolith structure

Etched transverse sections of otoliths from robust tongue sole displayed easily distinguishable rings instead of the opaque and translucent zones that are commonly found in many fishes. These rings were bright, with much higher transmittance than other areas of the otolith section. The high transmittance of the rings can be attributed to the acid etching step of our protocol. Two rings (concentric circles) and one ring formed at the margin of the otolith are shown in Fig. 2. In the SEM observation, the rings appeared to be deeply etched.

The bright ring structures of the robust tongue sole were formed seasonally. Fish with the bright zone at the otolith margin occurred at a high proportion ($> 70\%$) only in July–August (Fig. 3a). The GSI of males was relatively stable at a low level (< 1), but that of females showed a distinct seasonal fluctuation (Fig. 3b). Females developed gonads gradually, beginning in April, and gonads matured in June–July ($GSI > 3$). The GSI tended to decrease towards winter and did not exceed 2 between November and April. The spawning period was estimated to be mainly from June to August. This seasonality suggests that the tongue sole forms the otolith rings on an annual basis during the spawning period.

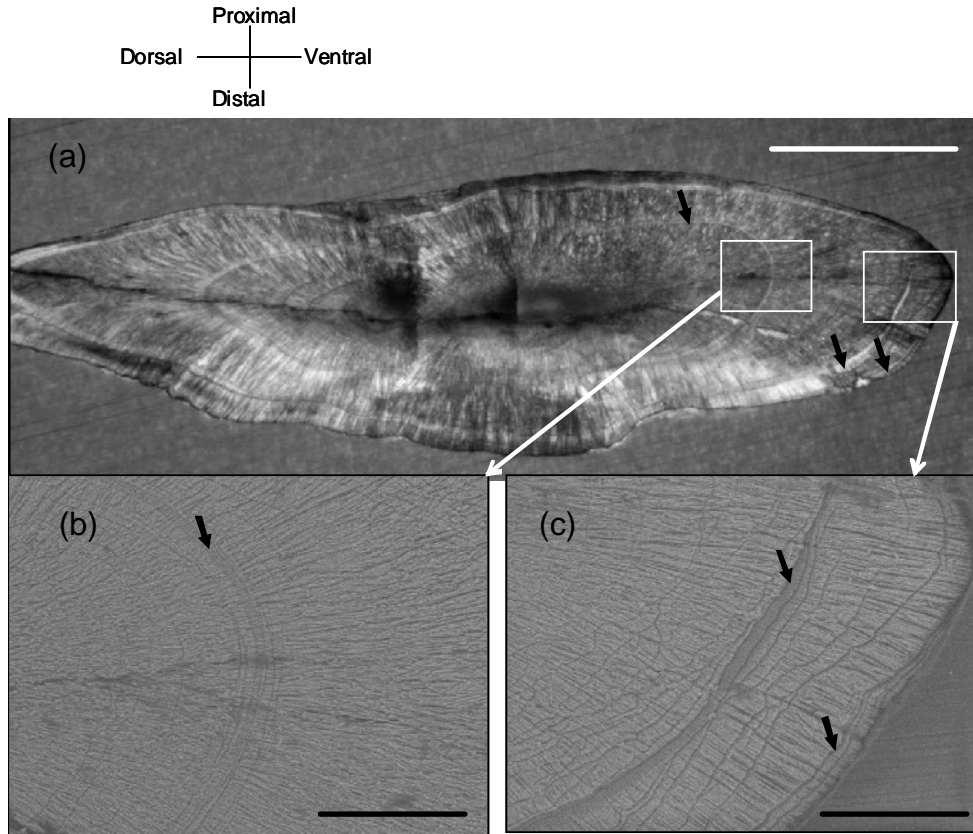


Fig 2. Otolith section of a 3 year old robust tongue sole (TL: 253 mm, Aug. 2000) showing the codification of the microstructural features considered in this study. The arrows indicate annual checks. Scale bars in (a) and (b, c) are 0.5 mm and 0.1 mm, respectively.

Age and growth

Individual age-length data was plotted to fit von Bertalanffy growth curves for males and females separately (Fig. 4). The sex ratio in the sample ($n = 642$) did not differ significantly from 1:1 ($z = 0.303, p > 0.05$). Maximum ages of male and female tongue sole were 11 and 7, respectively. Larger specimens (> 350 mm TL) were almost all female. Growth curves of male and female fish were estimated to be:

$$\text{Male: } TL_t = 324 \{1 - \exp(-1.16 t)\} + 1.8$$

$$\text{Female: } TL_t = 351 \{1 - \exp(-1.05 t)\} + 1.8$$

These formulae differed significantly between sexes ($F = 29.0, p < 0.05$), indicating that females become slightly larger than males.

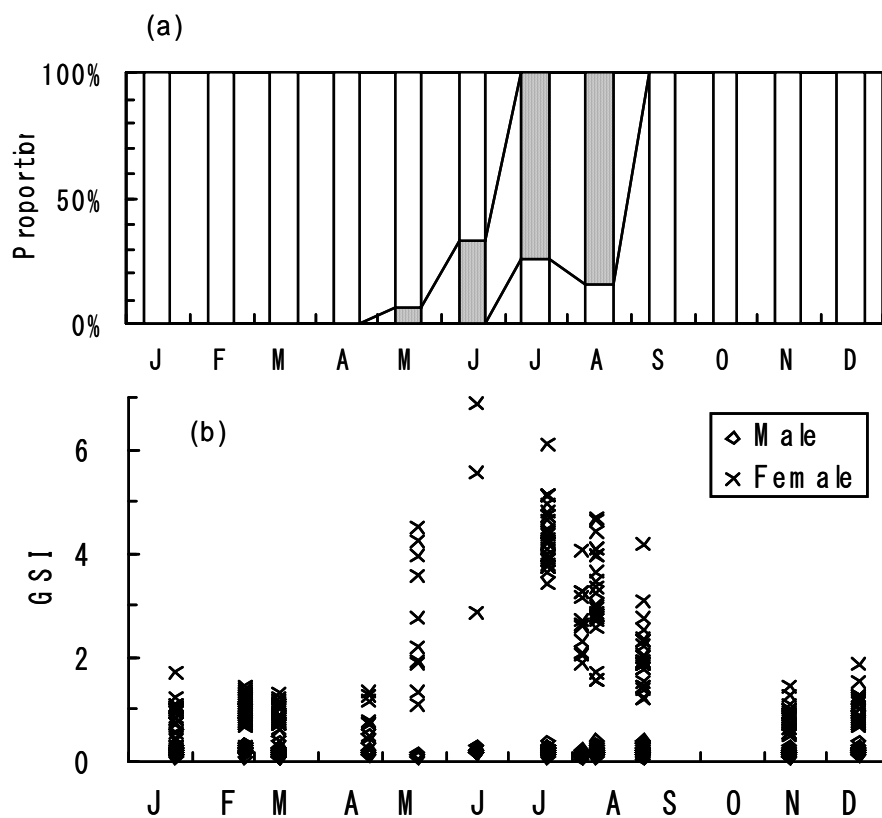


Fig 3. Seasonal changes in the proportion of each otolith marginal structure of robust tongue sole and in the GSIs of male (a) and female (b).

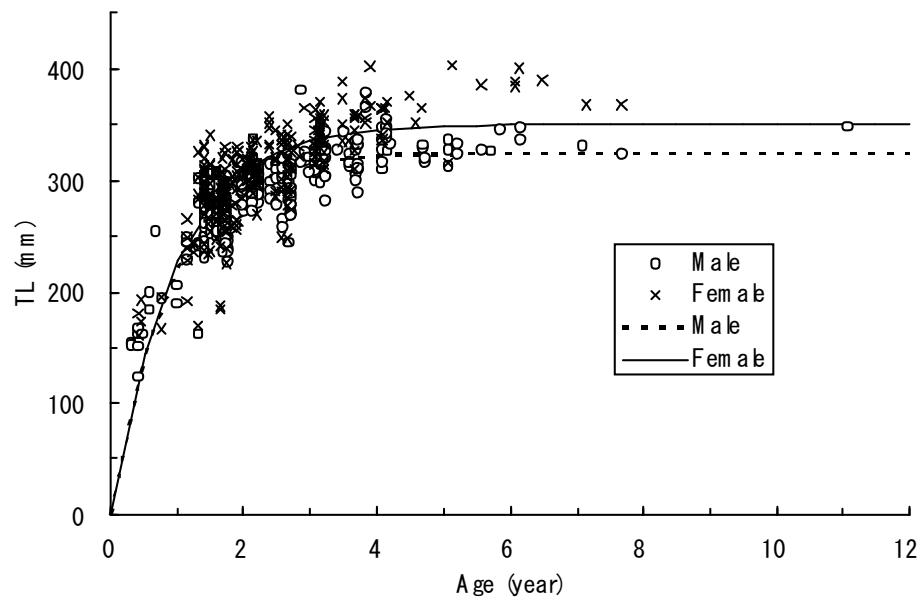


Fig 4. Age-TL relationships with von Bertalanffy growth curves for male and female of robust tongue sole

Maturation size and age

As described above, the GSI of males did not exceed 1 (Fig. 5a). Almost all females < 300 mm in TL had a GSI < 1, just like the males. Females matured after reaching 300 mm in TL. The female GSI-age relationship indicated that females matured at 2 years of age (Fig. 5b). The length-weight relationship is expressed as follows:

$$BW = 5.00 \cdot 10^{-7} \cdot TL^{3.44} \quad (r = 0.961, n = 642)$$

Relation of BW to TL is shown in Fig. 6. The age composition of the fisheries catch was ages 0–7. Ages 1–3 constituted about 90% of total catch abundance (Fig. 7a); therefore, fishery recruitment appears to be complete by age 1. Relative numbers to recruitment of each age were calculated as shown in Fig. 7b. Using BW-TL relation, relative weight of each age of the current status and the estimates without fishing mortality is shown in Fig. 7c. Assuming that female maturation rates were 100% at age 2 and that the sex ratios for each age class were 50:50, %SPR was calculated as total weight of shaded bars (current status) divided by total weight of light bars (estimates without fishing mortality) as shown in Fig. 6c, and found to be 30.1% for the robust tongue sole.

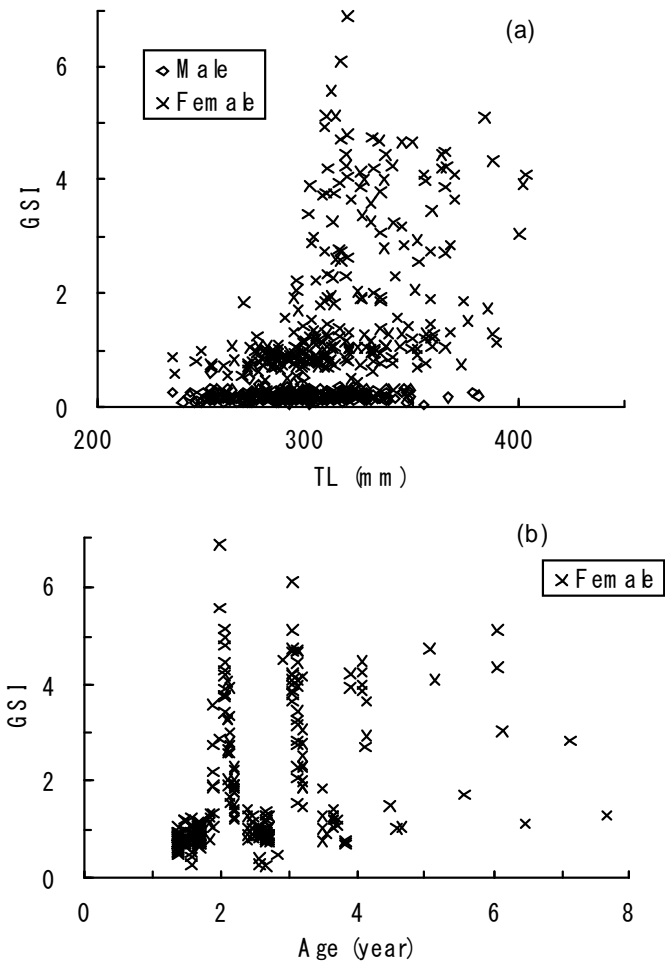


Fig 5. Relations of GSI to TL (a) and age (b) of robust tongue sole.

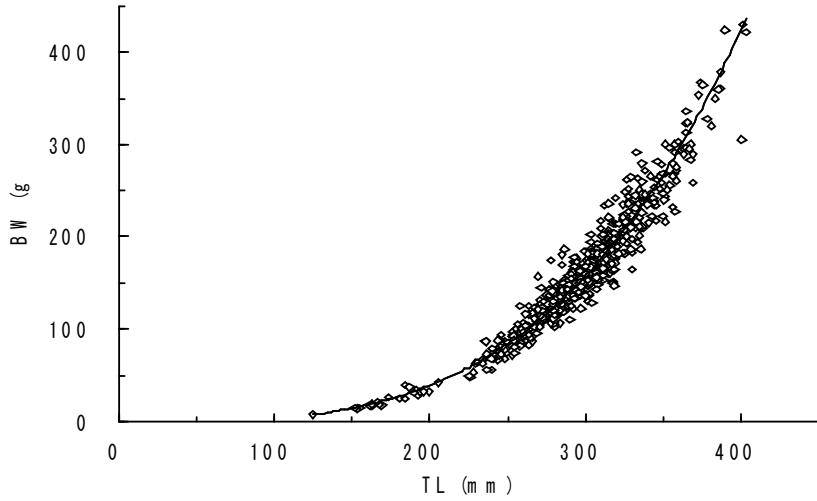


Fig 6. Relation of BW to TL of robust tongue sole.

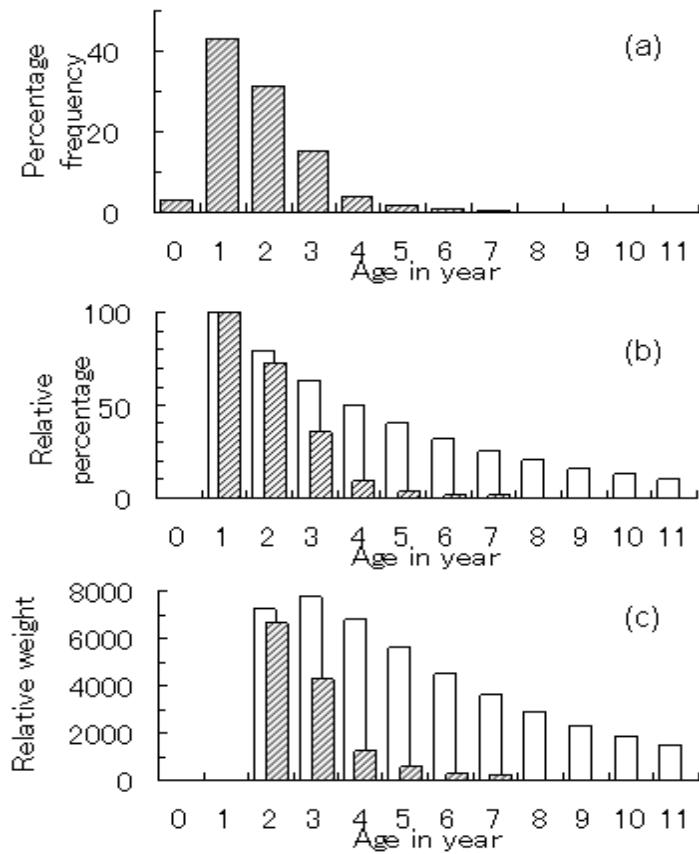


Fig 7. Age compositions of fisheries catch (a), relative numbers to recruitment of each age (b), and relative weight of matured female of each age (c) of robust tongue sole. Shaded and light bars indicate current status and estimates without fishing mortality, respectively.

Discussion

The sectioning and etching methods developed in the present study were powerful tools for identifying annual checks in otoliths of the robust tongue sole. After acid etching, structural discontinuities, or checks, are breaks that generally appear as deep grooves in the otolith section; under the microscope they appear as dark zones (Wright et al. 2002). The bright rings present in otoliths in our study correspond to checks, although they were bright rather than dark after etching. After acid etching, checks often are stainable by certain histological dyes (Albrechtsen, 1968; Pannella, 1980). The check is considered to be related to activity patterns and different life strategies (Morales-Nin, 1987), settlement and sex change (Munday et al. 2009), and stress (Campana, 1983). A check might also correspond to a seasonal ring, such as that induced by winter stress, and such checks can be useful for estimating annual age (Wright et al. 2002). The checks observed in robust tongue sole otoliths are formed during the spawning season; this result agrees with the premise of a spawning check proposed by Gauldie (1987). However, the first ring is not a spawning check but rather a check present in immature fish of less than 2 years old. Thus, physical changes that occur during the spawning season affect the structural discontinuities of otoliths, irrespective of whether or not the fish is a spawner.

In this study, seasonality of the check formation in otoliths was examined along with seasonal changes in GSI. Although females displayed an obvious seasonal fluctuation in GSI during the spawning season, a fluctuation in the male GSI was not detectible. Kusakabe (2011) reported the same phenomenon for this species. In the future, histological observations of the maturation process of male tongue sole should be made and compared with results from females. The present results on the age-TL were similar to those reported by Kusakabe (2011) for the Osaka Bay. However, the growth curve parameters for male and female were slightly different, i.e. $L_{\infty} = 353$ mm and 407 mm and $k = 0.70$ year⁻¹ and 0.64 year⁻¹ in Osaka Bay, whereas $L_{\infty} = 324$ mm and 351 mm and $k = 1.16$ year⁻¹ and 1.05 year⁻¹ of the present study, respectively. Larger k and smaller L_{∞} presented in this study were caused by utilising the biological intercept method that all growth curves passed through the origin of coordinates ($t = 0$ and $TL = 1.8$), and t_0 being zero. Growth trajectories of the fish in the central part of Seto Inland Sea (present study) and in Osaka Bay which is an inlet located easternmost of the Seto Inland Sea were found to be non-heterogeneous.

The present study also estimated age composition of fishery catches of the robust tongue sole from the Seto Inland Sea, Japan, using the newly developed otolithometric method. The age analysis showed a %SPR of 30%. A 30% SPR is needed to maintain the stock at its present level if

recent recruitment patterns persist (Gabriel et al. 1989). Demersal fishes inhabiting the Seto Inland Sea experience high fishing pressure due to the bottom trawl fishery (Katayama et al. 2010). The fast growth until the maturation age presented in this study would maintain the population, even under high fisheries activity.

Conclusion

An ageing methodology of sectioning and etching was developed which displayed easily distinguishable annual checks. Using this ageing method, age and growth of robust tongue sole could be documented for male and female. Besides that, the age composition of the fisheries catch was found to be ages 0–7. Ages 1–3 constituted about 90% of total catch abundance and the percent spawning potential ratio (%SPR) was estimated as 30%. Convenient and accurate method of age determination has made it possible to assess the population status and fishing pressure of the fish.

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