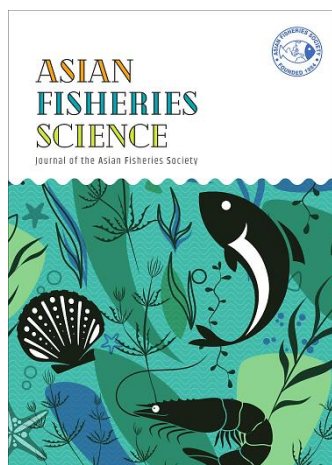




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Comparison of Age and Growth of the Marbled Flounder *Pseudopleuronectes yokohamae* (Günther, 1877) in the Coastal Waters of Japan

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Abstract

The marbled flounder, *Pseudopleuronectes yokohamae* (Günther, 1877), is one of the most economically important flatfish species in Japan. Research has been carried out on the age and growth of this species. However, there has been no comparison of growth rates of the fish from different locations. In this study, the age composition and growth trajectory of marbled flounder from different coastal waters in Japan were estimated and compared. Samples were collected from six locations, namely in the prefectures of Kagawa, Yamaguchi, Chiba, Niigata, Miyagi and Hokkaido. Age composition and growth trajectories were determined by analysing otoliths. The majority of specimens were found to be 2 to 3 years of age. Female marbled flounder were found to have a higher total length (TL) and growth rate than males. There were significant differences in growth curves for each of the sampling locations. There were also significant differences between males and females at each sampling location, with females attaining a higher theoretical maximum TL, longer lifespan and faster growth rate than males. The data from the current study can be used to help improve fish resource management.

Keywords: otolith, fish, age, growth

Introduction

Flatfish comprise an important part of global marine fisheries, representing 2.1 % of total fish weight landed and 1.6 % of commercial fishery value (FAO 2016). In Japan, marbled flounder, *Pseudopleuronectes yokohamae* (Günther, 1877) is one of the most commercially important flatfish species.

The marbled flounder is a demersal fish, found on coastal sandy and muddy bottoms. This species is widely distributed in Japanese waters, from southern Hokkaido to Oita Prefecture, and is also caught in the Yellow Sea, the Bohai Sea and the northern part of the East China Sea (Lee et al. 2009b).

Age and growth can be used to estimate important parameters, such as growth rate and longevity, which can reflect the growth trajectory of marbled flounder.

Comparing these parameters among different areas can also help in understanding how different environmental factors influence the growth of fish. Otoliths are an important tool in fish age determination. Otoliths are crystalline structures composed of calcium carbonate, contained in an endolymphatic sac of teleost fishes. They are mechanical components of the sound transduction mechanism responsible for hearing in fishes (Gauldie et al. 1990). The age structure and longevity of a fish species is important in comparing age and growth among various water bodies. The age of marbled flounder is usually determined by interpreting and counting growth rings on the whole otoliths (Kume et al. 2006; Lee et al. 2009a). Generally, the largest of three types of otoliths, the sagitta, which is contained in the endolymphatic sac, is used for analysis (Gauldie et al. 1990).

There are reports of significant differences in the weight-length relationship exhibited by male and female marbled flounder (Jawad et al. 2017). Analysing the parameters of the weight-length relationship by sex, values for males were higher than females (Dagang et al. 1992) and growth of females was faster than that of males (Kim et al. 2015).

There have been numerous studies on the age and growth of marbled flounder from different locations in Japan (Kooka et al. 2000; Tanda et al. 2008; Kume et al. 2006). However, there has been little research comparing the age and growth of marbled flounder from different waters to reveal the life history traits in each area. This study aims to compare the age and growth of *P. yokohamae* from coastal waters around Japan using otoliths, to investigate whether there are differences between locations, and to determine what factors are responsible for such differences.

Materials and Methods

Sampling area and sample collection

A total of 3344 specimens were collected to compare the age and growth of *P. yokohamae* from six different locations along the coastal waters of Japan (Fig. 1). The specimens were collected by commercial fishing vessels using either bottom trawl net (BT Net), bottom set net (BS Net) or the gill net (G Net) as indicated in Figure 1. The year the samples were collected is also shown in Figure 1.

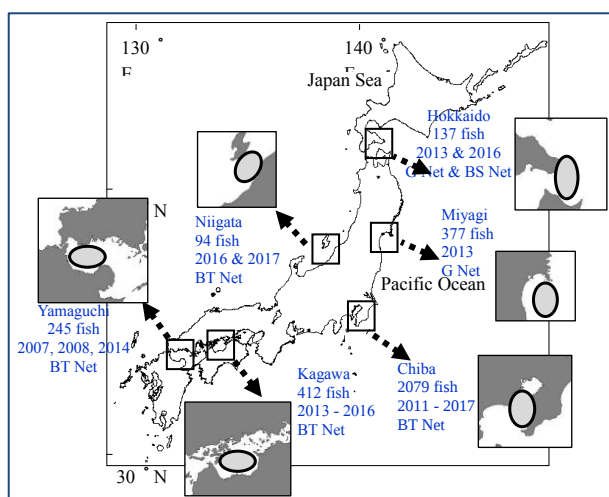


Fig. 1. Map of Japan showing the location of the sampling sites where *Pseudopleuronectes yokohamae* were collected, the number of specimens collected, the year and the type of net used. The location sites were namely prefectures of Yamaguchi (Suo Nada, Seto Inland Sea), Kagawa (Hiuchi Nada, Seto Inland Sea), Chiba (Tokyo Bay), Niigata (Sado Strait), Miyagi (Sendai Bay) and Hokkaido (Kikonai Bay). BT Net (bottom trawl net), G Net (gill net).

The total length (TL) of each specimen was measured to the nearest millimetre, without correcting for shrinkage, and the sex of each specimen was determined by visual examination of gonads (Kooka et al. 2000). The sagittal otoliths of *P. yokohamae* were dissected out, cleaned with distilled water, and stored dry in well plates until further analysis.

Otoliths observation and age determination

The sagittal otoliths were observed using the surface reading method to determine the age of *P. yokohamae* specimens. Otoliths become thick during periods of somatic growth, making the annual structures (annuli) invisible when using the surface reading method. For otoliths with unclear annuli, the sectioning method was used.

The sagittal otolith from the blind side of fish is generally better for age determination when using the surface reading method, because the innermost opaque region is located in the centre of the otolith, and the annuli are more visible here than that on the otolith from the ocular side (Polat et al. 2001). There were some losses of otolith samples from the blind side of specimens, however, there was no difference in the number of opaque rings between otoliths from the blind and ocular sides, and so otoliths from both sides were analysed using the surface reading method (Lee et al. 2009a). The growth annulus was defined as the opaque zone (Fig. 2).

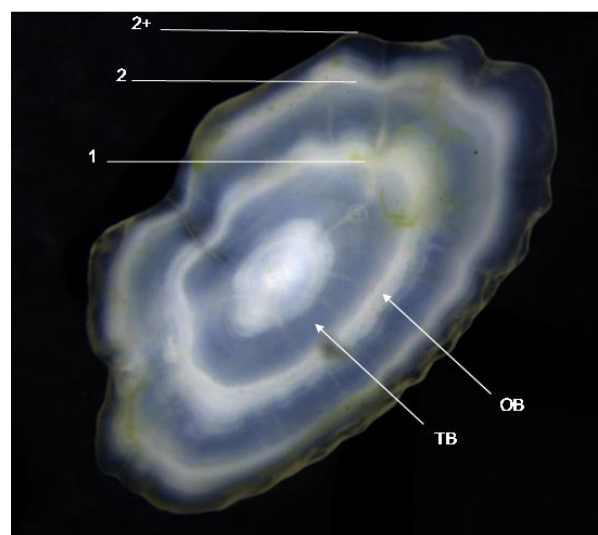


Fig. 2. Otolith collected from a marbled flounder *Pseudopleuronectes yokohamae* at the age of 2 + years, showing the numbers of annulus (years), translucent band (TB) and opaque band (OB).

For the sectioning method, otoliths were selected from fish that were more than 4 years of age. These otoliths were put on clay and filled with polyester resin. Each resin block contained about 30 otoliths. Resin blocks were sectioned transversely, with

sections running through the core of each otolith. Each section was mounted on a glass slide using wax and ground with sandpaper. Similar to the surface reading method, the opaque zone was regarded as the growth annulus. The otoliths were observed under reflected light on dark paper using an encoded stereomicroscope (M165C, Leica Microsystems, Germany). A digital camera attached to the microscope was used to take images of the otoliths for further analysis.

For the determination of age, the percentage of individuals with an opaque zone at the otolith edge for each month was determined, to confirm if the otolith annuli were formed annually. To accurately determine the age of *P. yokohamae* specimens, the age of all individuals was assumed to increase by one year on January 1st (Kume et al. 2006), and the age of each specimen was then calculated using the following equation:

$$t = O + \frac{(M-1) + \frac{D-1}{30}}{12}$$

where t is the age (in years), O is the number of opaque zones on the otolith, M is the month of sampling, and D is the date of sampling.

To analyse the growth of *P. yokohamae* from each sampling location, a von Bertalanffy growth curve was fit to the age-length data, using the equation:

$$L_t = L_{\infty}(1 - e^{-K(t-t_0)})$$

where L_t is the total length at age t , L_{∞} is the asymptotic length, K is the growth coefficient, and t_0 is the hypothetical age when total length would be 0. In this study, t_0 was set to 0 for all analyses, because t_0 widely tends to fluctuate with body size composition. The parameters of the von Bertalanffy growth equation were estimated using Solver on MS-Excel 2016 (Microsoft, USA), which implements a quasi-Newtonian method for nonlinear least-squares parameter estimation (Tokai 1997). Based on this method, the von Bertalanffy growth equations for individuals from each sampling location were provided for females and males separately.

Data analysis

The von Bertalanffy growth curves were compared among locations and within populations by gender, to compare the age and growth of *P. yokohamae* from different coastal waters. The F-test was used to test for differences in growth equation among locations and between sexes using MS-Excel 2016 (Microsoft).

Results

Otolith margin and annulus formation

There was no significant difference in the ratio of the otoliths with opaque edges between sexes, so the otolith margins of both sexes were analysed together. The percentage of individuals with an opaque zone at the otolith margin changed seasonally (Fig. 3). The opaque zone at the outer margin of the otolith appeared from December and peaked in April. Few individuals collected between June and December were recorded with opaque zones at the otolith margins. This suggested that the opaque zone is formed only once a year, coinciding with the spawning season of *P. yokohamae* around Japan (December–April). Therefore, the opaque zone was regarded as the annulus for age determination.

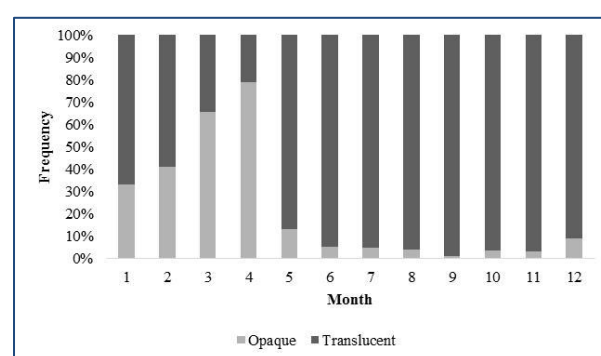


Fig. 3. Monthly changes in percentage occurrence of opaque and translucent zones at otolith edges of *Pseudopleuronectes yokohamae*. Samples of males and females were combined.

From these results, the region from the otolith kernel to the inner margin of the first opaque zone was assumed to represent one year of age, the region from the inner margin of the first opaque to inner margin of the second opaque zone represented two years old, and so on.

Age and total length compositions

Total length (TL) frequency distribution was made for each coastal water using 25 mm intervals (Fig. 4a–f); different colours represent the age of fish.

The age-total length composition is different in fish collected from the different coastal waters (Fig. 4a–f). Figure 5a–f showed that the total length frequency distribution and maximum body size were also different.

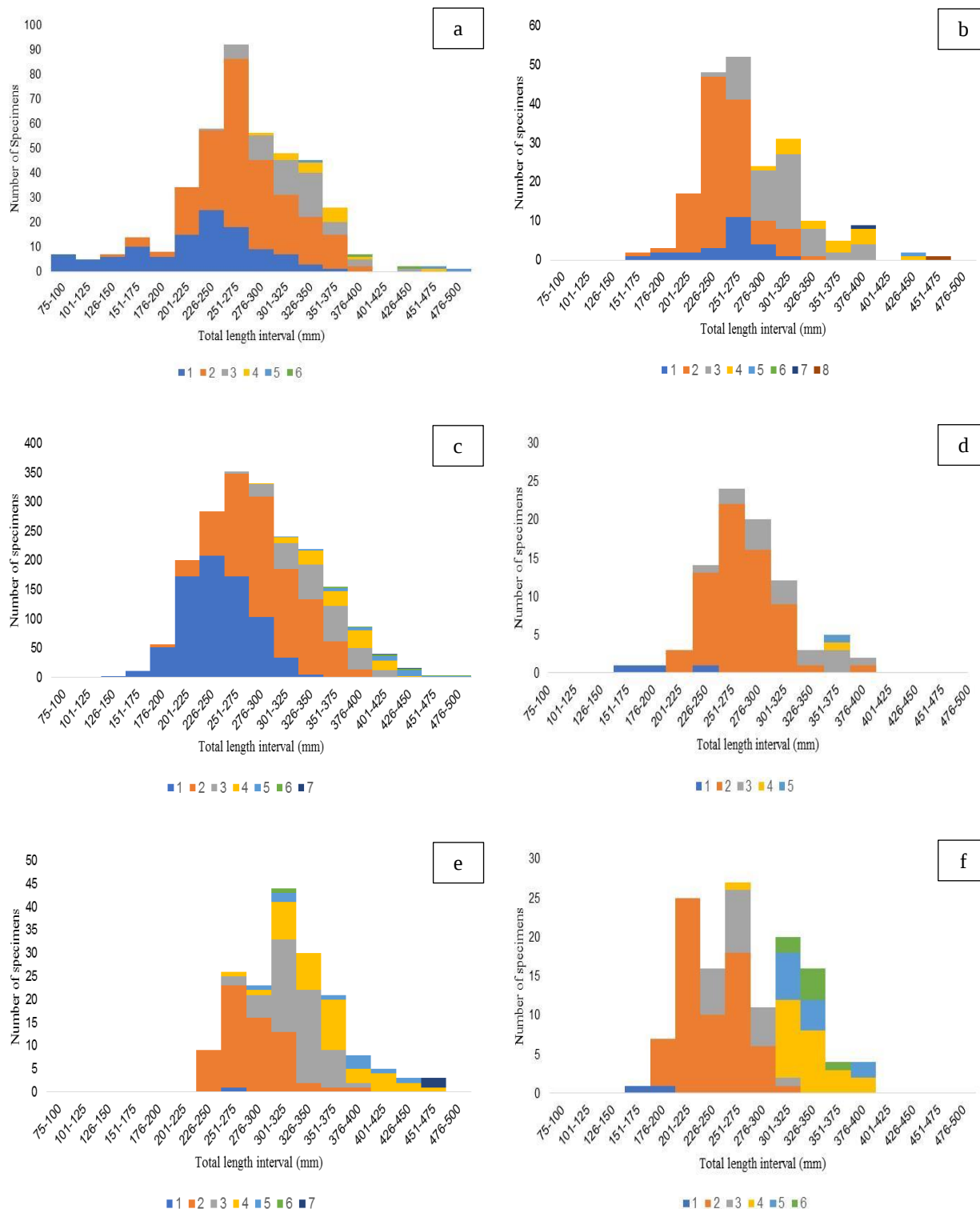


Fig. 4. Age and total length composition of *Pseudopleuronectes yokohamae* sampled from a) Kagawa Prefecture, b) Yamaguchi Prefecture, c) Chiba Prefecture, d) Niigata Prefecture, e) Miyagi Prefecture, and f) Hokkaido.

The fish specimens collected from the six locations vary in the body length, as shown in Figure 4a–f. The minimum length was 89 mm, and the maximum was 544 mm and ranged from 1 to 8 years in age.

Fish with a minimum length of 89 mm were from Kagawa Prefecture (Fig. 4a), whereas the bigger fish with a maximum length of 544 mm were from Miyagi

Prefecture (Fig. 4e). Although fish from Yamaguchi Prefecture were the oldest age group (8 years) the maximum length was 453 mm (Fig. 4b), which is smaller than those with the maximum age group of 7 years with a maximum length of 485 and 544 mm from Chiba and Miyagi Prefectures respectively (Fig. 4c, e).

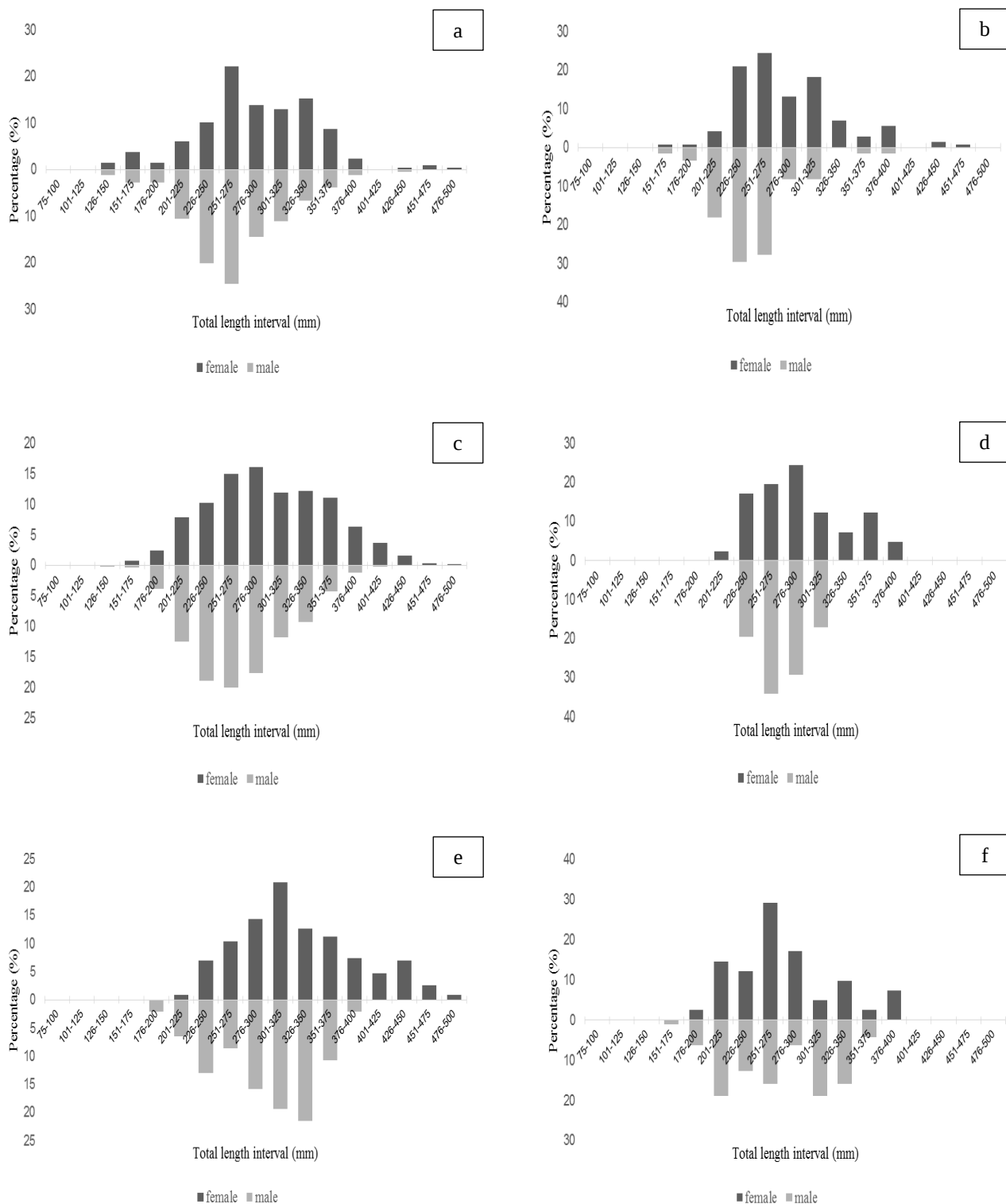


Fig. 5. Length-frequency distribution of male and female *Pseudopleuronectes yokohamae* sampled from a) Kagawa Prefecture, b) Yamaguchi Prefecture, c) Chiba Prefecture, d) Niigata Prefecture, e) Miyagi Prefecture, and f) Hokkaido.

From all six coastal waters, the majority of specimens were estimated to be 2 and 3 years of age, with very few individuals recorded over 7 years old. The majority of individuals were within the 200 to 350 mm size range. For specimens collected from Kagawa Prefecture, the males ranged in size from 145 to 428 mm with the common size from 251–275 mm and were the lowest percentage (22 %) compared to the other

locations (Fig. 5a). Whereas for the females the lowest percentage (21 %) was from Miyagi and ranged in size from 203–544 with a common size of 301–325 (Fig. 5e). The highest population of male and female were sampled from Niigata Prefecture with the size of the male of 232–320 and common size of 251–300 at 64 %, while the female population ranged from 222–386 with the common size from 226–300 at 61 % (Fig. 5d).

Growth curves and formulas

The relationship between TL and age can be fitted using a von Bertalanffy growth model, and therefore, the Solver method on MS-Excel was used to estimate TL at each age (Table 1).

Table 1. Predicted total length (mm) using the back-calculate method at each estimated age of *Pseudopleuronectes yokohamae* sampled from each sampling area.

Predicted length (mm) at each estimated age						
Estimated age	Kagawa			Yamaguchi		
	Both sexes	Female	Male	Both sexes	Female	Male
1	167.0	170.8	162.9	156.8	157.5	158.6
2	262.8	271.0	253.0	249.2	252.9	244.8
3	317.7	329.8	302.8	303.6	310.7	291.7
4	349.2	364.3	330.4	335.7	345.7	317.2
5	367.2	384.5	345.6	354.5	366.9	331.0
6	377.6	396.4	354.0	365.6	379.7	338.6
7	383.5	403.3	358.7	372.2	387.5	342.6

Predicted length (mm) at each estimated age						
Estimated age	Chiba			Niigata		
	Both sexes	Female	Male	Both sexes	Female	Male
1	175.4	179.7	173.9	136.9	131.4	166.8
2	272.5	282.4	262.4	228.3	226.9	243.7
3	326.4	341.0	307.3	289.3	296.3	279.2
4	356.2	374.5	330.2	330.0	346.7	295.5
5	372.7	393.7	341.8	357.2	383.3	303.0
6	381.9	404.6	347.7	375.3	409.9	306.5
7	386.9	410.9	350.7	387.4	429.2	308.1

Predicted length (mm) at each estimated age						
Estimated age	Miyagi			Hokkaido		
	Both sexes	Female	Male	Both sexes	Female	Male
1	148.4	140.1	170.5	117.4	114.7	116.8
2	244.8	239.9	257.3	201.2	201.1	199.6
3	307.3	310.8	301.5	260.8	266.1	258.2
4	348.0	361.3	324.0	303.4	315.0	299.7
5	374.4	397.2	335.4	333.7	351.9	329.0
6	391.6	422.8	341.3	355.3	379.7	349.9
7	402.7	440.9	344.2	370.7	400.6	364.6

The von Bertalanffy growth equations and curves for both sexes and the two sexes separately derived from the predicted TL were as follows (Fig. 6a–f):

Kagawa Prefecture (Fig. 6a): ($F = 1.44$ $P < 0.01$)

Female: $L_t = 413.2 (1 - e^{-0.533t})$

Male: $L_t = 364.46 (1 - e^{-0.592t})$

Yamaguchi Prefecture (Fig. 6b): ($F = 1.45$ $P < 0.05$)

Female: $L_t = 399.45 (1 - e^{-0.501t})$

Male: $L_t = 374.52 (1 - e^{-0.609t})$

Chiba Prefecture (Fig. 6c): ($F = 1.74$ $P < 0.01$)

Female: $L_t = 419.22 (1 - e^{-0.560t})$

Male: $L_t = 353.79 (1 - e^{-0.680t})$

Niigata Prefecture (Fig. 6d): ($F = 3.89$ $P < 0.01$)

Female: $L_t = 480.57 (1 - e^{-0.320t})$

Male: $L_t = 309.46 (1 - e^{-0.774t})$

Miyagi Prefecture (Fig. 6e): ($F = 0.39$ $P < 0.01$)

Female: $L_t = 485.77 (1 - e^{-0.340t})$

Male: $L_t = 347.29 (1 - e^{-0.675t})$

Hokkaido (Fig. 6f): ($F = 0.95$ $P < 0.05$)

Female: $L_t = 464.26 (1 - e^{-0.284t})$

Male: $L_t = 400.34 (1 - e^{-0.345t})$

For all populations, there were differences in growth between females and males, with the female fish showing higher growth rate, maximum total length and asymptotic age than males.

Discussion

Increment formation of otolith and age determination of fish

The results of this study showed that the opaque zone of the marbled flounder otolith was formed once a year in all of the sampling locations. The first translucent zone appeared around the end of the spawning season as confirmed in otolith observation of this study. Therefore, the outer margin of the translucent zone was regarded as the annulus for age determination. The data showed that there was no significant difference in the growth pattern in otoliths of marbled flounder collected from the different sampling locations. The results were similar with those for marbled flounder from other locations, and for other flatfish species (Tomiya et al. 2016; Jawad et al. 2017). In the present study, it was assumed that all specimens spawned on January 1st. To accurately determine the spawning season of marbled flounder, the daily growth increments of otoliths from juvenile marbled flounder should be investigated.

Age and growth of marbled flounder

In the current study, females were found to have greater longevity and TL than males at all six sampling locations. Solomon et al. (1987), Tanda et al. (2008), Shafieipour et al. (1999) and Kim et al. (2015) reported the same results from studies in Japan and Korea. Similar results have also been recorded for other flatfish species, including southern flounder, *Paralichthys lethostigma* Jordan & Gilbert, 1884, and blackfin flounder, *Glyptocephalus stelleri* (Schmidt, 1904) (Fischer et al. 2004; Yang et al. 2012).

The growth curves for specimens from different coastal waters around Japan (Fig. 7a, b), suggest that marbled flounder in Miyagi Prefecture and Niigata Prefecture showed the greatest L_{∞} and growth rate,

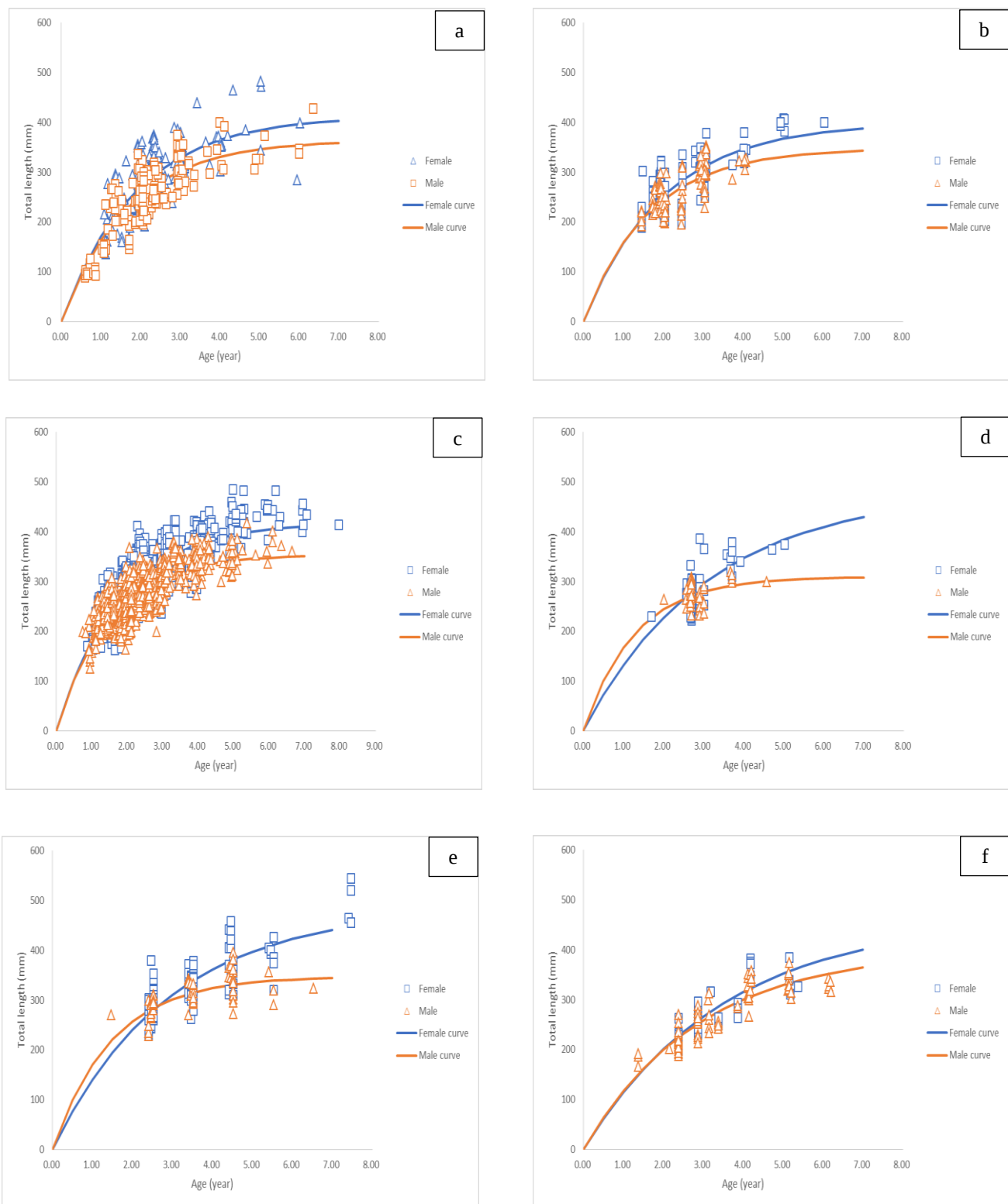


Fig. 6. Von Bertalanffy growth curve for male and female *Pseudopleuronectes yokohamae* sampled from a) Kagawa Prefecture (female: $n = 214$; male: $n = 179$), b) Yamaguchi Prefecture (female: $n = 146$; male: $n = 98$), c) Chiba Prefecture (female: $n = 1097$; male: $n = 978$), d) Niigata Prefecture (female: $n = 41$; male: $n = 41$), e) Miyagi Prefecture (female: $n = 63$; male: $n = 124$), and f) Hokkaido (female: $n = 41$; male: $n = 95$).

while male fish in Niigata showed the lowest growth among these 6 areas, and Hokkaido male fish has the highest growth rate. Previous studies on age and growth of marbled flounder in Japanese waters found similar trends (Shafieipour et al. 1999). The growth of marbled flounder is mainly affected by water

temperature, which can influence feeding and growth. The food consumption rate of marbled flounder generally increases with water temperature but decreases below 5 °C or above 25 °C (Kooka et al. 2000). This indicates that water temperature is an important factor which may explain geographical

variations in growth rate. Fonds et al. (1992) and Iwata et al. (1994) reported that water temperature has a similar effect on European flounder, *Platichthys flesus* (Linnaeus, 1758) and Japanese flounder, *Paralichthys olivaceus* (Temminck & Schlegel, 1846). Kooka et al. (2000) reported that L_{∞} was 415 and 362 mm for female and male fish, respectively, in Hokkaido between 1989 and 1991 (Shafieipour et al. 1999). In the current study, L_{∞} values from Hokkaido were 464 and 400 mm for female and male fish, respectively. The lengths recorded in the current study are greater than those of previous studies. There is a lack of environmental data in the previous studies for comparison with the present study. However, it is possible that global warming has led to the increased growth of marbled flounder recorded in this study.

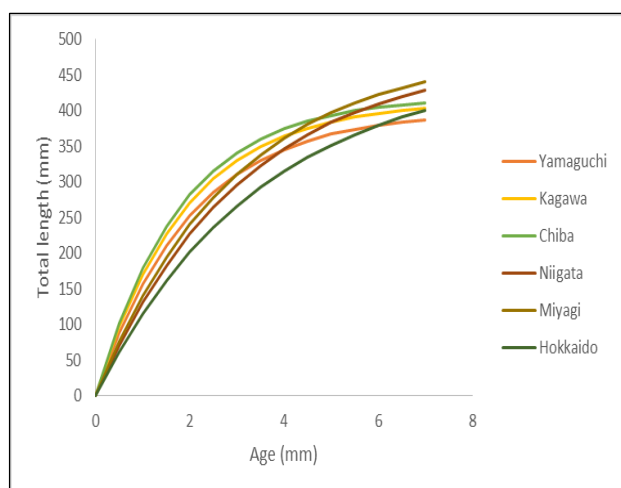


Fig. 7a. Comparison of von Bertalanffy growth curves for female *Pseudopleuronectes yokohamae* among different sampling areas.

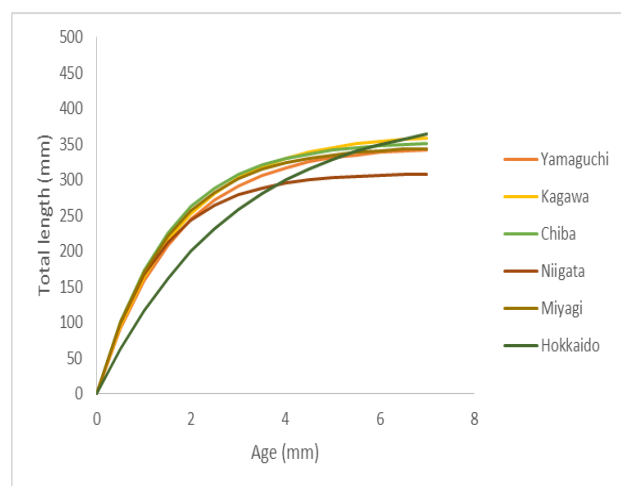


Fig. 7b. Comparison of von Bertalanffy growth curves for male *Pseudopleuronectes yokohamae* among different sampling areas.

As seen in Figure 4, TL and age composition show that in Miyagi Prefecture, no specimen was recorded under 200 mm, and almost all fish were over one year of age. In Miyagi Prefecture, a gill net was used for

sample collection. The smallest mesh size of the gill net is bigger than other fishing gears, which led to the difference in TL and age composition of marbled flounder from this location. It also suggests that fishing pressure in Miyagi Prefecture may be less than other areas.

Other than the different fishing gears used in sampling, stocking density also has great influence in the growth and size-frequency distribution. With the lack of population density data in each sea area, we chose catch per unit effort (CPUE) data as an index to reflect the population level of marbled flounder (Lee et al. 2009b). The CPUE in Seto Inland Sea is greater than that in Tokyo Bay, which indicates the population density in Seto Inland Sea is higher, and both in female and male fish. The growth in Chiba Prefecture is better than that in Kagawa and Yamaguchi Prefecture (Fig. 7a, b), which implied that the lower population density can offer a more optimum environmental condition for organisms. However, in other sea areas, the CPUE data was hard to compare together because of the different unit and effort of fishing gears.

In addition to the significant differences in the growth rate of marbled flounder in different coastal waters, the TL composition also showed differences. The proportion of small fish in Kagawa Prefecture and Yamaguchi Prefecture was higher than in other locations, and Chiba Prefecture had a higher proportion of younger fish than others. These three areas did not show a significant difference in average water temperature, in which the annual water temperature in the Seto Inland Sea is 10–27 °C, and in Tokyo Bay is 11–28 °C (water temperature data were downloaded from Japan Oceanographic Data Center https://www.jodc.go.jp/jodcweb/index_j.html), suggesting that the growth rate is related not only to water temperature, but also to pressure from fisheries. In Tokyo Bay (Chiba) and Seto Inland Sea (Kagawa and Yamaguchi), marbled flounder are subject to higher fishing pressure than other areas. Although the stock of marbled flounder in Miyagi Prefecture and Hokkaido seemed to be at an appropriate level, the growth parameter was lower than that reported in a 2004 study in Hokkaido (Shafieipour et al. 2004). Therefore, it is important for fishery management committees to develop a stock assessment model for the management of marbled flounder based on the age and growth of this species. In addition, to confirm the growth mechanism of the otoliths, further studies, including rearing experiments, are required.

Conclusion

In this study, otolith opaque zones of marbled flounder *P. yokohamae* form once a year, and mainly in April, which can be used in age determination and fit the von Bertalanffy growth equation. The growth

equations and curves suggest that the age and growth are different among the six sampling areas, and also show the difference between female and male fish within a population. Female fish in all the sampling areas has a greater growth than male. From the results in this study, factors that may influence the growth of fish such as water temperature, fishing pressure and abundance of food organisms should be investigated in the future, and the growth of *P. yokohamae* can also help in stock assessment of each sea areas in Japan.

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