



ISC/21/BILL WG-03/09

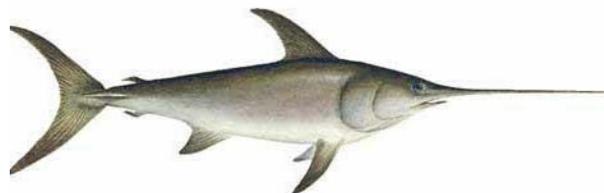
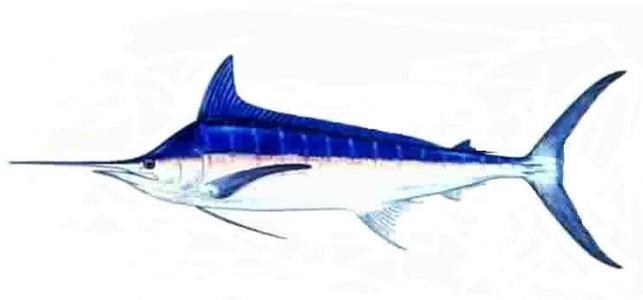
Preliminary results of daily age estimation for juvenile striped marlin (*Kajikia audax*) caught  
in the seas around Japan, North West Pacific Ocean

Miyuki Kanaiwa<sup>1</sup>, Ayumu Furuyama<sup>1</sup>, Hirotaka Ijima<sup>2</sup>, Akira Kurashima<sup>2</sup>, Minoru Kanaiwa<sup>1</sup>

<sup>1</sup>Faculty of Bioresources, Mie University, Mie, Japan

<sup>2</sup>Highly Migratory Resources Division Fisheries Stock Assessment Center Fisheries  
Resources Institute (FRI) Japan Fisheries Research and Education Agency

Email: k-miyuki@bio.mie-u.ac.jp



## **Abstract**

Striped marlin and swordfish differ in age and growth between the eastern and western Pacific, but the cause of this difference is not known. Since age estimation methods differed between the eastern and western Pacific studies, a unified approach is necessary. Therefore, at ISC/20/BILL WG-01/05, it was agreed that the daily age estimation should first be done using otoliths to determine the body length at one year of age, followed by age estimation using spines (Kanaiwa et al. 2020). The study followed that method to estimate the daily age of striped marlin using otoliths and compared the estimates of daily age between Kopf et al. 2011 and Shimose and Yokawa 2019, which used the same method to estimate the daily age of striped marlin. The 95% confidence interval widened significantly with the addition of this study. Although the results were not necessarily consistent with Shimose and Yokawa (2019) and Kopf et al. (2011), there were no major inconsistencies. This inconsistency may be partly due to the small number of cross-reading sessions and the fact that there are issues with the reader's skill level and reading style. Therefore, the present results are preliminary and will be recounted again.

## **Introduction**

We compared the growth of marlin species in the eastern and western Pacific Ocean in ISC/20/BILLWG-1. It was suggested that the striped marlin (*Kajikia audax*) and swordfish (*Xiphias gladius*) caught in the western Pacific Ocean grew faster (DeMartini et al., 2007, Kopf et al., 2011) than those caught in the eastern Pacific Ocean (Sun et al., 2002, Sun et al., 2011). The largest difference in eye-fork length (EFL) between eastern and western Pacific striped marlin was at age 3 for both males and females, at approximately 32.1 cm and 37.3 cm, respectively. For swordfish, the largest difference was about 40.0 cm for females at age 5 and 37.2 cm for males at age 6. However, since age estimation methods differed between the eastern and western Pacific studies, age estimation using a unified method is necessary. Therefore, it was agreed at ISC/20/BILLWG-01 that, when conducting age estimation, daily age estimation using otoliths should be conducted to determine the body length at one year of age, followed by age estimation using spines (Kanaiwa et al. 2020). The purpose of this study is to show the preliminary relationship between daily age and EFL of striped marlin in the seas around Japan and to compare it with the relationship in the eastern Pacific Ocean shown by Shimose and Yokawa (2019). In addition, since some countries use LJFL when measuring the length of striped marlin, a new conversion factor between LJFL and EFL for juvenile striped marlin was developed.

## **Materials and Methods**

Otoliths from a total of 19 marlin individuals, 4 males, 11 females, and 4 unidentified (Figure 1 and Table 1), were caught in the waters around Japan (Figure 2 ) between August 24, 2019, and June 9, 2021. The head of the striped marlin was collected and frozen after measuring the eye-fork length (EFL). Males and females were identified by observation of the genital gland. The EFL of the striped marlin used in this study ranged from 110.6 to 180.1 cm. The primary method of daily age estimation was based on Shimose and Yokawa (2019). The thawed heads were split in the laboratory using a saw according to the open-the-hatch method (Long and Grabowski 2017), and otoliths, including otic capsules, were collected. Collected otoliths were defleshed using tweezers and dissecting needles and stored in 2.0 ml microtubes with 75% ethyl alcohol.

These otoliths were embedded in epoxy resin (Type 53, PELNOX), and sections of approximately 300  $\mu\text{m}$  were obtained using a precision cutting machine (IsoMet<sup>®</sup>Low speeds, BUEHLER). The sections were glued to glass slides with the thermoplastic resin (CRYSTALBOND 509-1, AREMCO). The obtained preparations were polished using a polishing cloth (2TS4, MARUTO) and alumina powder (Baikalox<sup>®</sup> PRECISION ALUMINUM OXILE 0.3CR<sup>®</sup>, BAIKOWSKI) in a tabletop polishing machine (ML-110NT, MARUTO). Polished otoliths were photographed using an optical microscope (OLYMPUS BX53), a digital microscope camera (AS ONE HDCE-20C), and ScopeImage 9.0 image reading software. The images were captured under a magnification of 100x with a 1600 x 1200 pixels capture size and saved in jpeg format. The photos were combined using the image editing software gimp (2.10.18ver.) and saved in PNG format. The composite photos were used to assess the daily age using imageJ Fiji (imageJ-win64) image processing software, and each daily ring was recorded with a sequential number (Figure 3). Two daily ring readers counted all otoliths once each, and the average value was used as the daily age (Figure 4, 5 and Table 2). This result was added to the results of Shimose and Yokawa (2019) and Kopf et al. (2011) to obtain a linear equation for the logarithm values of the number of daily rings and of body length as a conversion factor. In this study, the body length in Kopf et al. 2011 and Simose and Yokawa 2019 was converted to EFL (Low-jaw fork length). For the conversion from LJFL to EFL, we used the striped marlin caught between 2016 and 2021, for which we had LJFL and EFL data (Figure 6 and Table 3). Using Equation 1, the point estimation and 95% confidence interval of the EFL for a 365-day-old, i.e., 1-year-old, striped marlin, were determined.

## Results and Discussions

The EFL and number of daily rings of the 19 individuals used in this study are shown in Table 2. This study estimated individuals ranging from 155 to 335.5 days old. The LJFL to

EFL conversion equation for young striped marlin was estimated as follows (Figure 7):

$$\text{EFL} = 0.885 \times \text{JFL} - 5.8664 \quad \text{Equation 1}$$

$$R^2 \text{ value} = 0.986$$

These observed values were plotted against the estimates of Kopf et al. (2011) and Shimose and Yokawa (2019), which converted body length from LJFL to EFL (Figure 7 and 8). The following equation was calculated from the age and EFL data in this study and previous studies (Kopf et al. 2011, Shimose and Yokawa 2019: Figure 9).

$$\ln(\text{EFL}) = 0.41335 \times \ln(\text{daily age}) + 2.52452 \quad \text{Equation 2}$$

As a result, the EFL of the striped marlin was estimated to be 143.1 cm when it was one year old. The 95% confidence interval was 137.3 cm to 149.0 cm, and the predicted value was 109.5 cm to 187.0 cm (Table 4).

The relationship between length and age in days when the observations of this study are removed (data B) is shown in Figure 10 and Table 5. The equation of daily age and EFL in data B is as follows:

$$\ln(\text{EFL}) = 0.39773 \times \ln(\text{daily age}) + 2.56228 \quad \text{Equation 3}$$

The 95% confidence interval widened significantly with the addition of this study. Although the results were not necessarily consistent with Shimose and Yokawa (2019) and Kopf et al. (2011), there were no major inconsistencies. The wider variety of estimated age may be partly due to the small number of cross-reading sessions and the fact that there are issues with the reader's skill level and reading style. Therefore, the present results are preliminary and will be recounted again.

### **Future work**

The daily age estimation of the samples used in this study will be conducted again to determine the body length per day and age. Microscopic observation was also used for areas where photographs could not confirm the daily rings, and each reader was counted three times. We will also increase the number of samples of otolith sections of marlin and increase the data for daily age assessment. The same sample processing and daily age assessment will be conducted for swordfish to determine the width of the EFL at age 1.

## References

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Figures

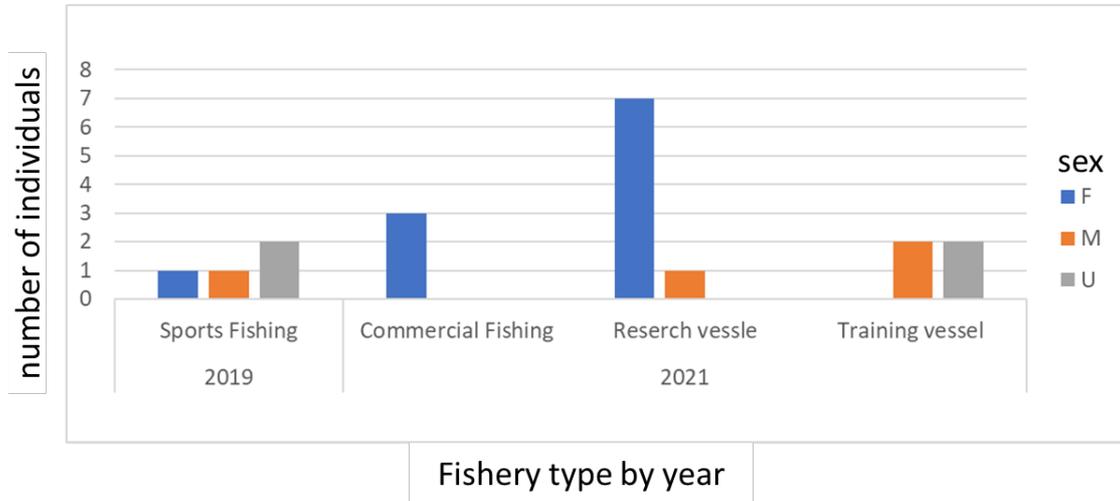


Figure 1 This figure shows the number of individuals by fishery type by year. Blue Indicate for female, orange for male and grey for unknown sex.

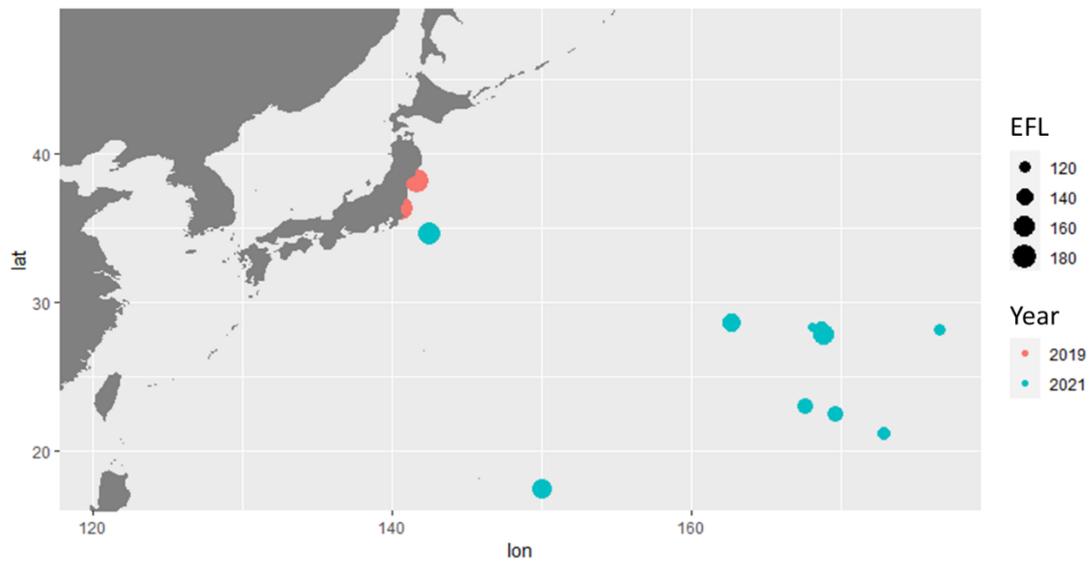


Figure 2 This map shows the locations where striped marlin was caught. Individuals caught in 2019 are shown in red and those caught in 2021 in blue. The size of the circles has been varied according to body size.

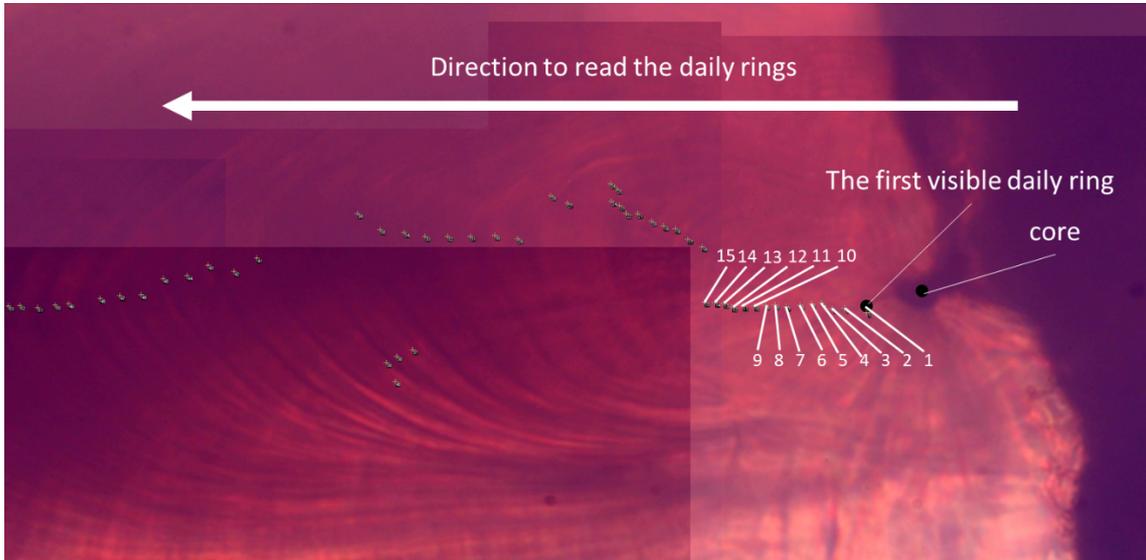


Figure 3 The black circle shows the core of otolith and the first visible daily ring. The images were recorded using ImageJ Fiji with sequential numbering of the daily rings.



Figure 4 The comparison of daily ages between reader A and B. The circles show the age data. The Dotted line is 45-degree line.

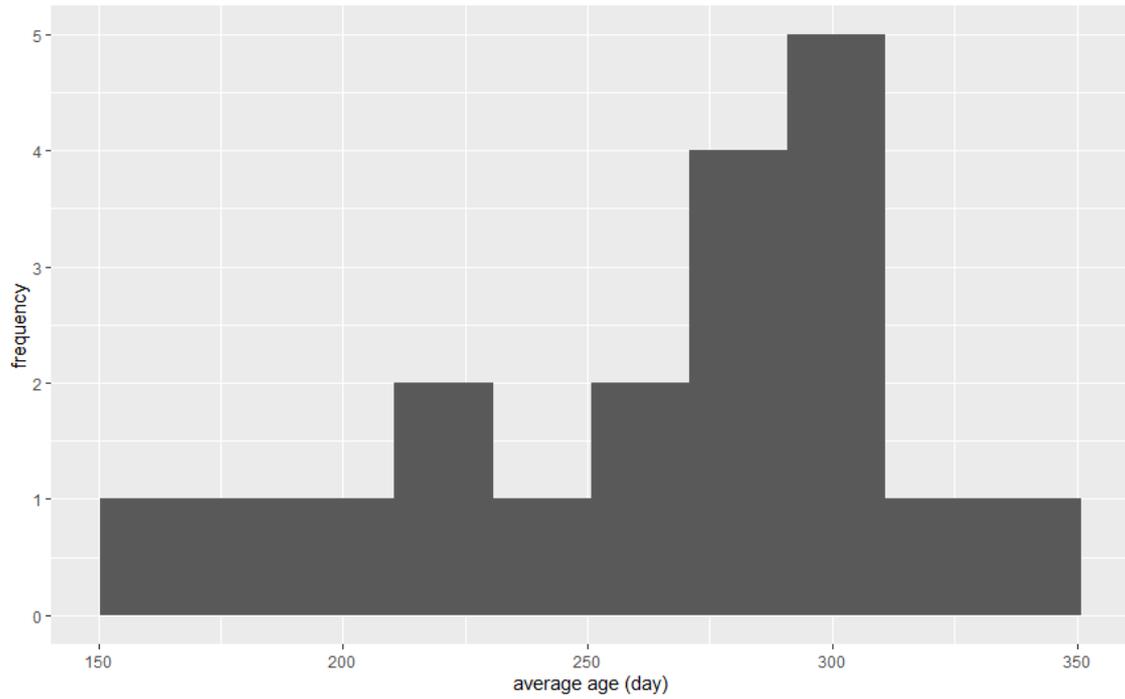


Figure 5 This figure shows a histogram of the relationship between observed daily age. The vertical axis shows the frequency and the horizontal axis the average of the daily ring counts of reader A and reader B.

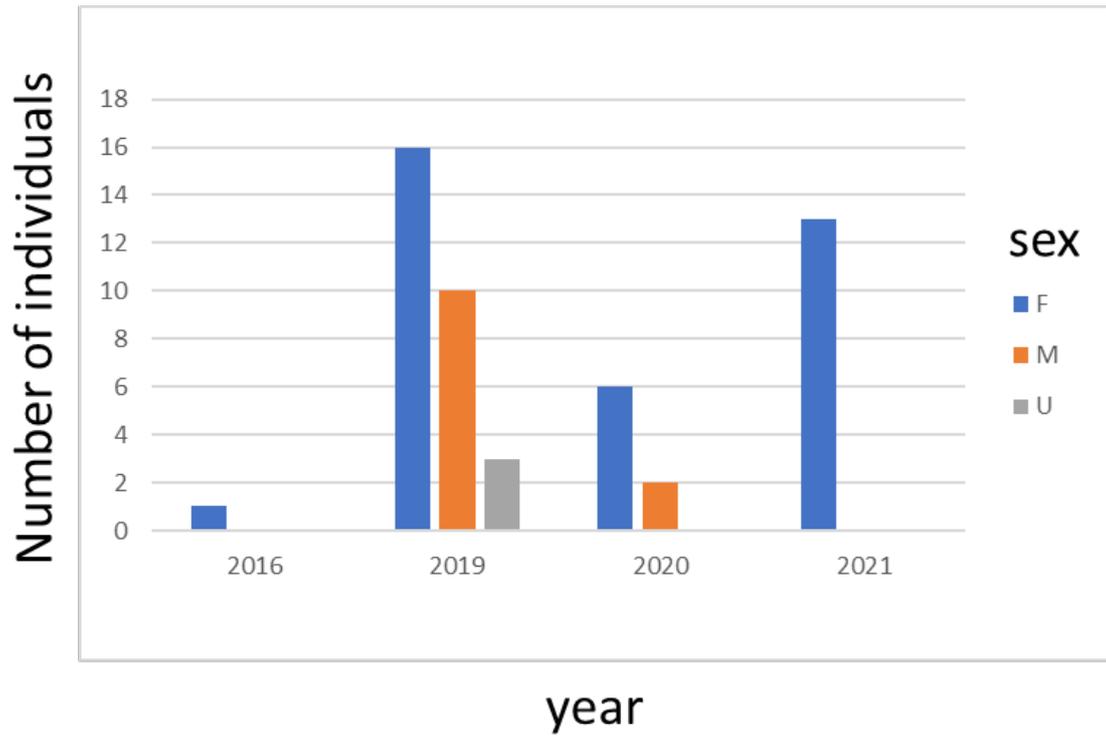


Figure 6 This figure shows a breakdown of the data used in the creation of the conversion factor from LJFL to EFL. The vertical axis and the horizontal axis represent the number of individuals and the year of catch, respectively.

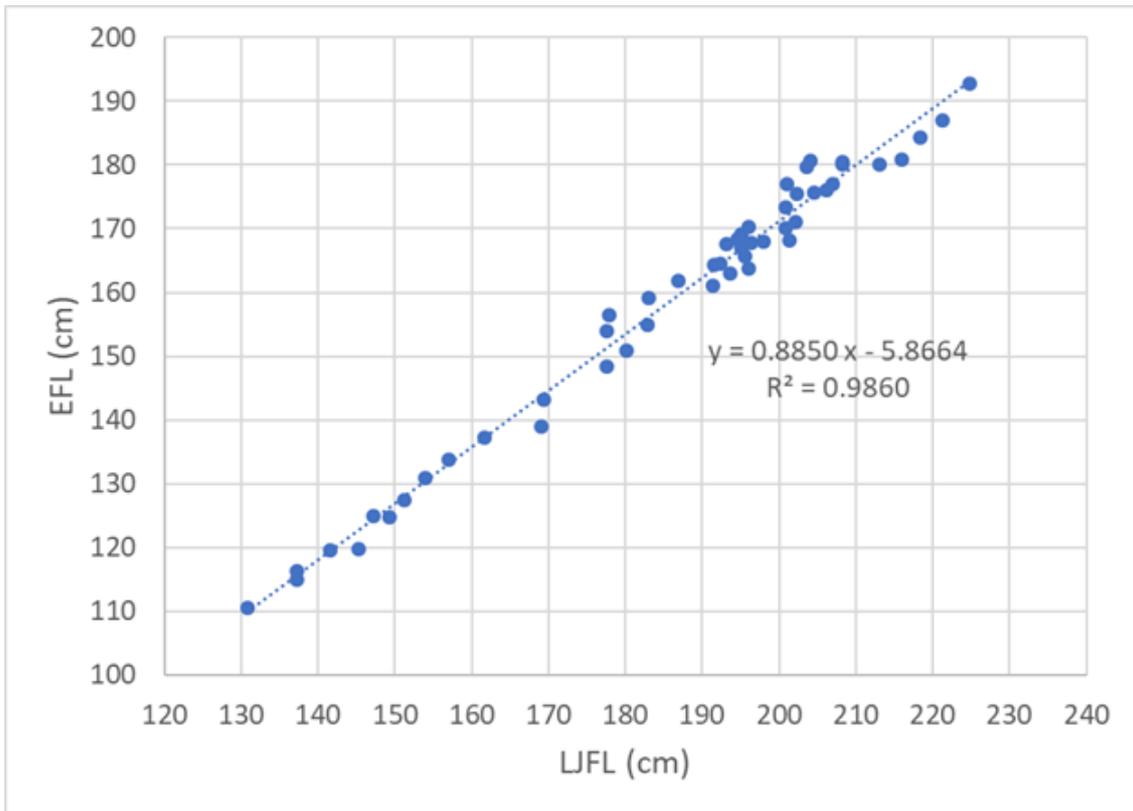


Figure 7 Using 51 striped marlin caught between 2016 and 2021 for which LJFL and EFL data were available, a conversion equation from LJFL to EFL was developed using a linear approximation.

The formula was  $y=0.8850x-5.8664$ ,  $R^2=0.9560$ .

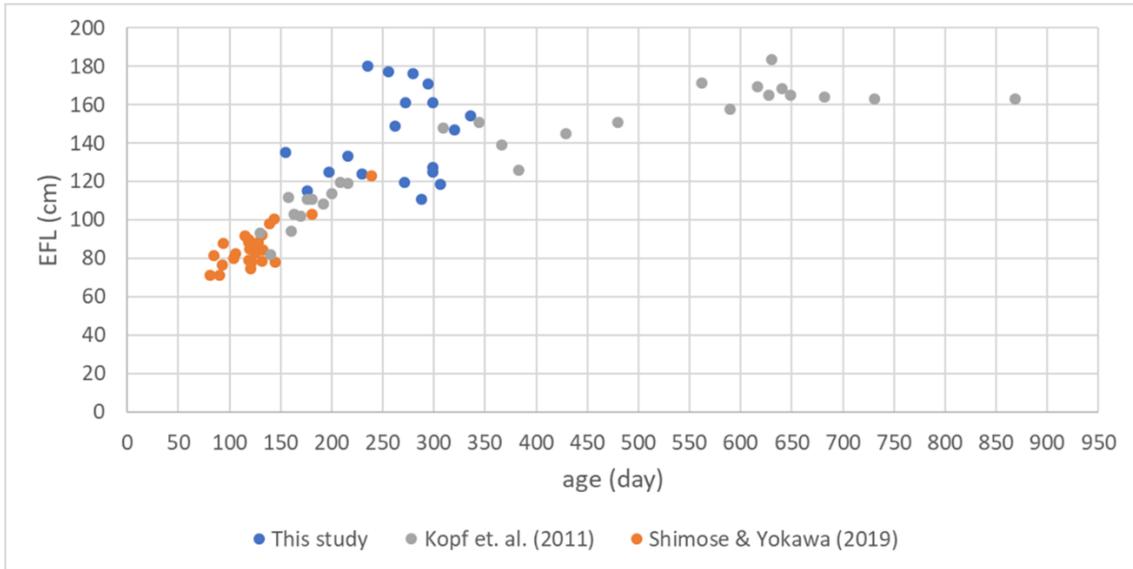


Figure 8 This figure plots the EFL-transformed Kopf et al. (2011) and Shimose and Yokawa (2019) estimates overlaid with the results of this study. The vertical axis shows EFL and the horizontal axis shows the age (day). The blue circles represent the results of the age assessment of this study, the grey circles represent the estimates of Kopf et al, (2011) and the orange circles represent the estimates of Shimose and Yokawa (2019).

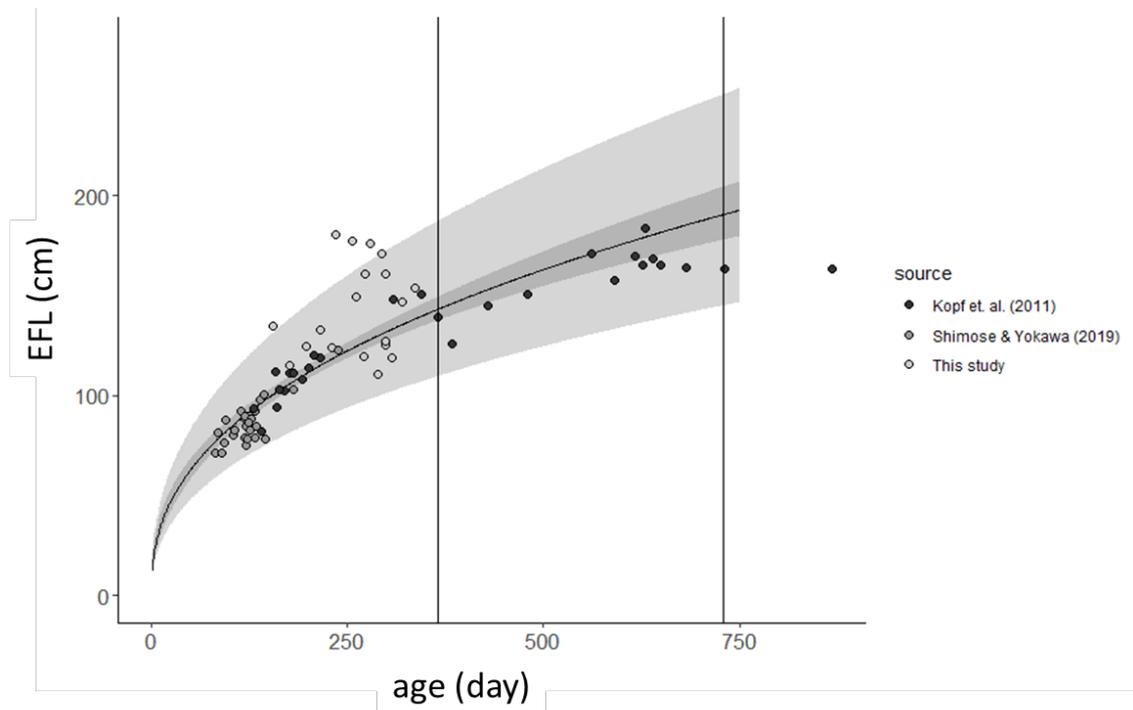


Figure 9 This figure is obtained by linear regression of EFL (cm) per age (day) when data A is used. The data used are the results of the daily age estimated in this study and the estimates of EFL (cm) per age (day) from Kopf et al. (2011) and Shimose and Yokawa (2019). The vertical axis represents EFL (cm) and the horizontal axis represents age (day).

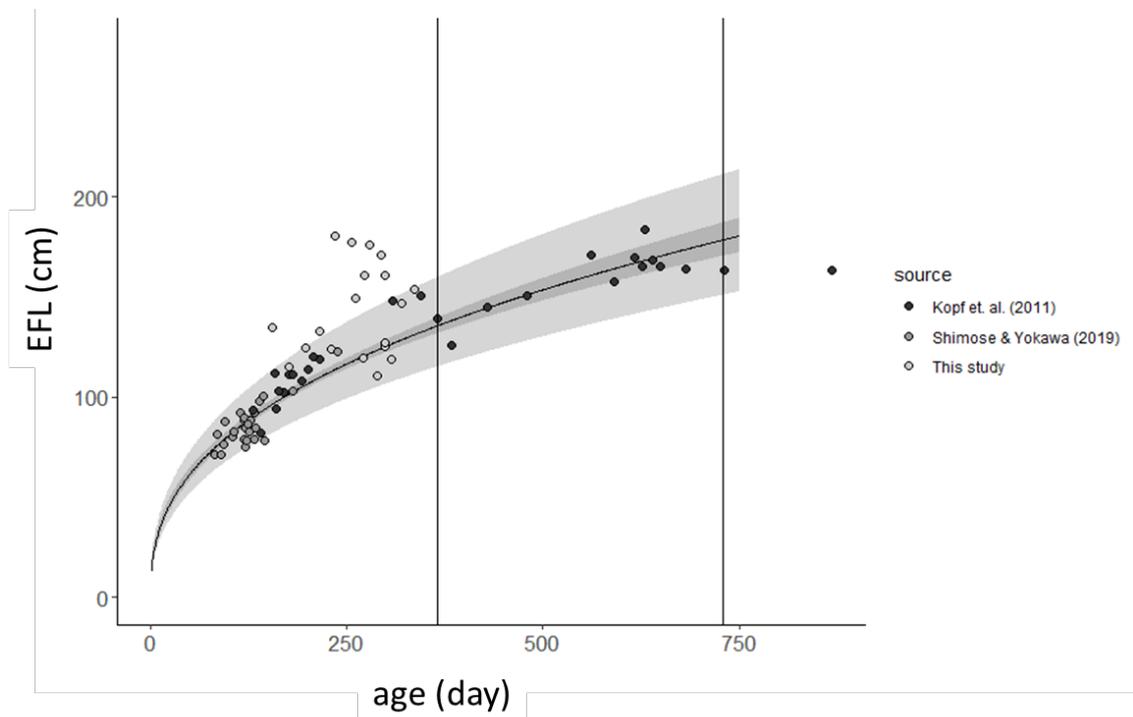


Figure 10 This figure is obtained by linear regression of EFL (cm) per age (day) when data B is used. The data used are the results of the daily age estimated in this study and the estimates of EFL (cm) per age (day) from Kopf et Al. (2011) and Shimose and Yokawa (2019). The vertical axis represents EFL (cm) and the horizontal axis represents age (day).

## Tables

Table 1 This table shows the number of individuals by year of catch, sex and fishery type. It also shows the minimum and maximum EFL (cm) by year of catch and by fishery type.

	Fishery type	F	M	U	Total	Min / EFL (cm)	Max / EFL (cm)
<b>2019</b>		1	1	2	4	161	180.1
	Sports Fishing	1	1	2	4	161	180.1
<b>2021</b>		10	3	2	15	110.6	171
	Commercial Fishing	3			3	110.6	119.8
	Reserch vessle	7	1		8	118.6	171
	Training vessel		2	2	4	124	154
<b>Total</b>		11	4	4	19	110.6	180.1

Table 2 This table shows the results of the daily ring counts for reader A and reader B in this study. In this study, reader A and reader B were each counted once. Column 12 shows the average of the daily ring counts for reader A and reader B.

No.	ID	Species	Sex	Catch date	Fishery type	EFL	reader A	reader B	Count error	Error ratio	Average	Hatch date
1	STM1907	STM	M	24 August 2019	Sports Fishing	176	272	286	-14	5.0179	279	25 November 2018
2	STM1912	STM	F	31 August 2019	Sports Fishing	177	251	260	-9	3.5225	255.5	23 December 2018
3	STM1924	STM	U	1 September 2019	Sports Fishing	161	322	275	47	15.745	298.5	14 October 2018
4	STM1925	STM	U	1 September 2019	Sports Fishing	180.1	251	220	31	13.163	235.5	24 December 2018
5	BF4713	STM	U	18 February 2021	Training vessel	124	223	236	-13	5.6645	229.5	10 July 2020
6	BF4716	STM	M	20 February 2021	Training vessel	135	157	153	4	2.5806	155	16 September 2020
7	BF4719	STM	U	21 February 2021	Training vessel	133	229	202	27	12.529	215.5	07 July 2020
8	BF4723	STM	M	27 April 2021	Training vessel	154	368	303	65	19.374	335.5	24 April 2020
9	BF4729	STM	F	22 April 2021	Reserch vessle	149	274	249	25	9.5602	261.5	22 July 2020
10	BF4734	STM	F	26 April 2021	Reserch vessle	118.6	323	289	34	11.111	306	07 June 2020
11	BF4752	STM	M	26 April 2021	Reserch vessle	147	325	316	9	2.8081	320.5	05 June 2020
12	BF4753	STM	F	26 April 2021	Reserch vessle	161	301	243	58	21.324	272	29 June 2020
13	BF4773	STM	F	28 April 2021	Reserch vessle	124.8	218	176	42	21.32	197	22 September 2020
14	BF4778	STM	F	28 April 2021	Reserch vessle	125	362	235	127	42.546	298.5	01 May 2020
15	BF4779	STM	F	28 April 2021	Reserch vessle	127.4	329	268	61	20.436	298.5	03 June 2020
16	BF4802	STM	F	2 May 2021	Commercial Fishing	119.8	283	259	24	8.8561	271	23 July 2020
17	BF4804	STM	F	22 May 2021	Commercial Fishing	110.6	295	281	14	4.8611	288	31 July 2020
18	BF4814	STM	F	9 June 2021	Reserch vessle	171	301	287	14	4.7619	294	12 August 2020
19	BF4819	STM	F	23 May 2021	Commercial Fishing	115	174	179	-5	2.8329	176.5	30 November 2020

Table 3 This table represents the 51 individuals caught between 2016 and 2021, and these 51 individuals have both LJFL and EFL data. I have used this data to create a conversion factor for EFL from LJFL.

	F	M	U	Total
2016	1	0	0	1
2019	16	10	3	29
2020	6	2	0	8
2021	13	0	0	13
Total	36	12	3	51

Table 4 This table shows the estimated EFL (cm) by daily age when data A is used. data A = All data, age Y = age of year, age D = age of day, EFL (cm), l = lowest of confidence, u = uppermost of confidence, pl = lowest of prediction, pu = uppermost of prediction,

data A						
age Y	age D	EFL	l	u	pl	pu
0.5	182.5	107.4204	104.021	110.9309	82.28892	140.2271
1	365	143.0602	137.3218	149.0384	109.4591	186.9761
1.5	547.5	169.1636	159.8363	179.0352	129.0615	221.7262
2	730	190.5245	177.731	204.2388	144.9278	250.4666

Table 5 This table shows the estimated EFL (cm) by daily age when data B is used. dataB = removed data of this study, age Y = age of year, age D = age of day, EFL (cm), u = uppermost of confidence, l = lowest of confidence, pu = uppermost of prediction, pl = lowest of prediction

data B						
age Y	age D	EFL	l	u	pl	pu
0.5	182.5	102.8408	100.5806	105.1518	87.38838	121.0256
1	365	135.4863	131.6266	139.4593	115.0085	159.6104
1.5	547.5	159.1963	153.1582	165.4724	134.8647	187.9176
2	730	178.4947	170.3721	187.0045	150.9084	211.1237

