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Reproductive traits of female skipjack tuna *Katsuwonus pelamis* in the western central Pacific Ocean (WCPO)

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Ohashi, S., Aoki, Y., Tanaka, F., Fujioka, K., Aoki, A. and Kiyofuji, H.

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Ohashi, S., Aoki, Y., Tanaka, F., Fujioka, K., Aoki, A. and Kiyofuji, H.

National Reserach Institute of Far Seas Fisheries, Japan Fisheries Research and Education Agency

Summary

1. Reproductive traits (sex ratio, spawning season, maturity size, batch fecundity, and spawning frequency) of female skipjack tuna in the WCPO were reconsidered based on histology.
2. The sex ratio was generally 1:1 over the whole WCPO.
3. In areas except for the temperate zone (<30° N), ovaries in the spawning capable phase were recognized at any time of the year, and in the temperate area (>30° N) it was recognized to appear from June to October (almost in July and August).
4. Minimum sizes at maturity were approximately 40 cm FL in all areas, however, the proportions of matured individuals in the 40 cm size class were less than 10%. The size at 50% maturity were remarkably different among the areas and were larger in the northern areas.
5. The batch fecundity increased with increasing body size. Relative batch fecundity tended to be larger in the southern area than the northern area.
6. Spawning intervals showed no large differences among the areas.

Introduction

Reproductive traits (sex ratio, maturity length and age, spawning season and area, and fecundity) are basic and very important biological information used to estimate spawning stock biomass (SSB) in stock assessment frameworks as well as for age determination and growth estimation. SSB is one of the most common criteria used as biological reference points for determining stock status for appropriate management action for related fisheries.

In the last stock assessment of skipjack tuna *Katsuwonus pelamis* in the western and central Pacific Ocean (WCPO) (McKechnie *et al*., 2016), it was assumed that the sexual maturity of skipjack tuna occurred uniformly at age-class 3 (7–9 months old), and the biomass of adult fish (age-class 3–16: 7–48 months old) was supposed to be an equivalent to the spawning stock biomass (SSB). These assumptions were based on the previous studies that fragmentally reported the appearance of mature individuals of skipjack tuna in the tropical WCPO (Matsumoto *et al*., 1984; Wild and Hampton, 1994). However, considering recent studies on the reproductive biology of skipjack tuna, such assumptions should be reconsidered.

The most recent research on the reproductive biology of skipjack in the WCPO based on histological analysis dates back to the 1970s (Asano and Tanaka, 1971). Although the authors of the research collected biological information including gonad around Palau and northern Mariana, there were not enough samples to specify the spawning seasons and other reproductive traits such as sex ratio. Similar work in recent years showed that the minimum sizes and sizes at 50% maturity of female skipjack tuna in the tropical WCPO were calculated to be 40.0 cm and 50.3 cm fork length (FL), respectively (Ashida *et al*., 2017). Schaefer and Fuller (2019) showed differences in size at 50% maturity of female skipjack tuna between three areas divided by latitude in the eastern Pacific Ocean. This evidence indicates that 40 cm FL is too small as the criteria of maturity size of female skipjack in the not only WCPO but throughout the Pacific. Understanding the reproductive dynamics and parameters of skipjack tuna in the WCPO has not been well described.

In this document, we describe the reproductive traits of female skipjack tuna in the tropical to temperate WCPO using a histological approach based on large-scale sampling conducted from 2006 to 2016. The information will be available for the skipjack stock assessment in 2019.

Material and Method

Two data sets were utilized in this study. One is detailed data including observation results of gonads, which was utilized for consideration reproductive traits such as the spawning season, maturity size, batch fecundity, and spawning frequency. The other is simple measurement data for verifying the sex ratio.

Information of ovarian samples

The dataset of 4,701 ovarian samples were utilized for characterizing the reproductive traits (spawning season, maturity size, batch fecundity, and spawning frequency) of fish caught in the tropical to temperate WCPO (11° S–42° N) by mainly commercial fishing fleet from 2003 to 2016 (**Fig. 1**).

Histological procedures

Procedures of making sectional samples were mainly based on that of Ashida *et al*. (2017). Ovarian tissues were fixed in 10% formalin, cut into small pieces, dehydrated in a series of ethanol concentrations, embedded in Paraplast (Sigma-Aldrich Inc. USA), sectioned at approximately 7μm thickness, and stained with Mayer's hematoxylin and 1% eosin (i.e., HE staining).

Ovarian maturity phases

In this study, the ovarian maturity phases were classified into four; immature, developing, spawning capable, and regressing. Ovaries in the immature phase consist of only unyolked oocytes (e.g., perinucleolus or yolk vesicle stage). The developing phase is characterized by the appearance of vitellogenic oocytes and has an intensity of atresia (percentage of α-atretic oocytes in all vitellogenic oocytes) of less than 50%. The spawning capable phase is indicated by the stage of germinal vesicle migration and spawning evidences (hydrated oocytes or postovulatory follicles), and the intensity of atresia to be less than 50%. Ovaries in the regressing phase include vitellogenic oocytes but lack any evidence of spawning, and the intensity of atresia is over 50%. Individual with ovary in any of the three phases (developing, spawning capable, and regressing) was recognized as a matured fish.

Batch fecundity

The batch fecundity was defined as the number of hydrated oocytes in the ovary in the spawning capable phase (Murua *et al*. 2003). Counting was performed by observation of three subsamples (0.05g in each) derived from different portions of the same ovary. Relative batch fecundity (RBF) was calculated by dividing batch fecundity by body weight minus gonad weight (g) in order to compare differences between areas excluding the influence of body size.

Maturation size

The relationship between maturity rate and size (FL in cm) was described by using the logistic equation, and parameters were estimated in the generalized linear model (GLM) with logit function and binominal distribution (0: immature; 1: mature) in R software (R core team, 2018).

Spawning fraction

The spawning fraction was calculated as a proportion of individuals classified as in the spawning capable phase relative to the total number of matured individuals (in developing, spawning capable, or regressing stage). The spawning interval was expressed as the inverse of the spawning fraction according to Hunter and Macewicz (1985).

Sex ratio

Sex ratios were verified based on the data of 57,527 skipjack tuna (27,021 males and 30,506 females) caught in the tropical to temperate WCPO (6° S–45° N) by Japanese research or training vessels from 1964 to 2017.

Area definition

In this study, the reproductive traits were compared latitudinally between different area definitions. Firstly, two areas were considered, north western Pacific and central western Pacific in the WCPO, which was divided on the 20° N line following the last stock assessment (Fig. 1a). Secondly, the areas were compared to smaller divisions (three areas: temperate (>30° N), subtropical (10° N–30° N), and tropical (<10° N)) (Fig. 1b).

Result and discussion

Sex ratio

The sex ratio of skipjack tuna was generally 1:1 in the WCPO which is shown as the proportion female in **Fig. 2**. The percentage female varied from 46.4 to 58.2% within the most common size range of 37 to 70 cm FL which consists 95% of the all samples from the whole area (**Fig. 2a**).

Sex ratio has been reported to be close to 1:1 in the Indian Ocean (Grande *et al.*, 2012), the Atlantic Ocean (Cayre *et al.*, 1986), and the tropical Pacific Ocean

(Ashida *et al*., 2017). This indicates that, based on the assumption of similar natural mortality and growth between female and male, only female SSB should be considered for making management recommendations. However, on the other hand, a recent histological study conducted in the eastern Pacific Ocean reported a slight bias towards male in large individuals (Shaefer and Fuller, 2019), which is inconsistent with previous research. In this case, sex-related growth equations should be considered since energy budgets relating to sexual maturity and growth were assumed to differ between males and females. It would need further examinations in the future assessment. It should be noted that data employed in this document were based on the observation of external morphology of gonads, not histological observation. In each area definition (**Fig. 2b–f**), biased sex ratios were also recognized in small and large size classes, and it was assumed that the small sample size in such size classes (each occupies less than 5% of the total) should have an impact on these results. Thus, a further examination of misspecification of sex in small individuals appears warranted, and it is recommended that future studies achieve sufficient number of samples to analyze for the large individuals.

<u>*Spawning season*</u>

Fig. 3 shows the monthly composition of maturity phases indicated by the sampled ovaries. In the area definition dividing the whole area into north western Pacific and central western Pacific at 20°N, the spawning capable phase was present in all months except for May in north western Pacific (>20°N). In the case of the area definition with three areas (temperate, subtropical, and tropical), the spawning capable phase was present all year round in the areas of subtropical and tropical. However, in the area of temperate, it was present only in June to September with the majority occurring in July and August (90.1%). Among the two area definitions, the areas of north western Pacific (>20°N) and subtropical (10–30°N) indicated that their percentages of individuals that in the spawning capable phase in May were remarkably lower than those of other months. A possible explanation for these results is the bias in the sampling locality. In May, no samples had been collected in the area between 18 and 29° N, and the proportion of samples collected in the area between 29° and 30° N in the area of subtropical (in the range of 10–30°N) was 92.6%. Therefore, the differences in May might be mainly due to the sampling bias.

It should be noted that the spawning season in the temperate area is shorter and limited to summer time, in comparison to the subtropical and tropical areas where the skipjack spawning occurs all year around. Spawning capable individuals were found in the area where sea surface temperature was ranged from 22.6 to 29.6°C. The range included temperature below the lower limit where skipjack larvae have been found (warmer than 24°C; Ueyanagi, 1969) and where the spawning capable females have previously been detected (Nishikawa et al., 1985). In addition to this evidence, potential skipjack spawning grounds were reported in the north Pacific Ocean over the summer period (Naganuma et al., 1972; Ashida and Horie, 2015). This implies that the high mobility of this species leads to the inhabitation of spawning capable females in the temperature lower than suitable for spawning, when considered their needs for feeding even during the spawning season.

Size at maturity

The minimum sizes at maturity were 38.2–40.9 cm FL and no drastic differences were observed among the areas (**Fig. 5; Tables 1–3**). This result seems to correspond with the assumption about maturity size in the last stock assessment (McKechnie *et al*., 2016). However, the proportions of mature individuals at the 40 cm size class were only 1.2–7.5%. In addition, those at each size class were remarkably different between the areas (**Figs 5 and 6; Table 3**). The relationship between FL and the maturity proportion in each area was given by the following logistic equations.

$$P_{\text{north western Pacific}} = 1 / (1+\exp(4.785 - 0.076\times \text{FL}))$$

$$P_{\text{central western Pacific}} = 1 / (1+\exp(8.080 - 0.163\times \text{FL}))$$

$$P_{\text{temperate}} = 1 / (1+\exp(2.403 - 0.043\times \text{FL}))$$

$$P_{\text{subtropical}} = 1 / (1+\exp(5.372 - 0.100\times \text{FL}))$$

$$P_{\text{tropical}} = 1 / (1+\exp(7.414 - 0.148\times \text{FL}))$$

Considering the sampling bias and spawning season based on the result mentioned in the section “Spawning season” (**Fig. 3**), samples caught in May were excluded from the analysis for the area of subtropical, and samples caught in July and August were utilized to represent the area of temperate. Size at 50% maturity in each area showed noticeable differences between the two or three areas, and the difference was larger in the northern areas (**Fig. 6; Tables 1–3**).

Size at 50% maturity is an important life history parameter that directly impacts the estimation of SSB estimation in the stock assessment. This size was estimated in Shaefer and Fuller (2019) with a definition of the maturity as a female having vitellogenic oocytes in its ovary. While, in the estimate of Grande *et al*. (2014)

was based on the observation of cortical alveolar oocytes as the definition of maturity, which indicated that skipjack in the western Indian Ocean would mature at smaller sizes than reported in the eastern Pacific Ocean and our research herein. The differences in size at 50% maturity between the eastern (Shaefer and Fuller, 2019) and the western Pacific (this study), and the western Indian Ocean (Grande *et al*., 2014) cannot be attributed to the definitions of maturity size since each study employed the same methods and difinitions.

Shaefer and Fuller (2019) employed the same methods as in our study and showed geographical variations of the skipjack size at 50% maturity which indicated isolation of the stock structures might had led to the size variation. In the western Pacific Ocean where our samples were collected, it has been revealed from tagging research that skipjack move from the tropical area toward the sea near Japan (Kiyofuji *et al*., 2019) and there is therefore mixing of fish between these areas to a certain extent (Phillips *et al*., 2018). Thus, the stock in the area is not assumed to be isolated as unlike the situation in the eastern Pacific Ocean. Skipjack migrate north with seasonal temperature increases, however, the relative temperature in the temperate area (corresponding to north western or temperate in this study) is low compared to the tropical and subtropical areas. The slightly larger size at 50% maturity estimated by this study may be caused by this temperature difference which requires longer to accumulate enough energy for maturity due to the low metabolic activity in the cool environment.

Batch fecundity and interval

The batch fecundity was calculated as 31,663–1,872,858 which increased with increasing body size (**Fig. 7**). Relative batch fecundity (RBF) tended to differ between the areas and be larger in the southern area than the northern area (**Fig. 8; Tables 1 and 2**). Although size-dependent fecundity would have an impact on the spawning potential because larger fish would produce relatively more eggs per unit body weight, the area and its environment also need to be taken into account for further analysis.

The spawning interval, which was expressed as the inverse of the spawning fraction, ranged between 1.93–1.97 days in the WCPO (**Tables 1 and 2**). No prominent differences were detected between the areas; however, the intervals were slightly shorter in the southern area compared to the northern area. These results are similar to those reported in the east Pacific Ocean when reproductively active females were used (Shaefer and Fuller, 2019). The method used to estimate the spawning interval assumed no migration among areas, which may be inaccurate for skipjack tuna in the WCPO, especially in northern areas. This is because spawning capable individuals may be influenced largely by the environmental factors in the northern area (e.g. duration of suitable temperatures for spawning activity) compared to the southern areas. In addition, it remains a challenge to clarify the total number of spawned eggs in a particular season due to longevity of postovulatory follicles after spawning (Hunter and Macewicz, 1985). This would have a substantial impact on not only estimates of reproductive parameters but also estimates of recruitments.

Conclusion

In the last stock assessment of skipjack tuna in the WCPO, the sexual maturity of skipjack tuna was assumed to occur uniformly at age-class 3 (7–9 months old). In this document, however, several results suggested a need to revise this assumption.

1. The spawning season differed among the areas, particularly in the area of temperate (>30° N) which was almost restricted to the summer season (July and August) (**Fig. 3; Tables 1 and 2**).
2. The proportions of mature individuals at the 40 cm size class, corresponding to age-class 3, were less than 10% in all areas (**Fig. 5**), and the size at 50% maturity were remarkably different among the areas (49.4–63.3 cm FL). Hence, the occurrence of sexual maturity is not uniform and the regional differences in the whole WCPO should be considered.

Moreover, the assumption used in previous stock assessments might lead to an overestimation of the spawning stock biomass (SSB). Therefore, other settings for the maturity parameters need to be tested, using the size at 50% maturity estimated in this study.

Supplemental information

Additional information on estimating the size at 50% maturity is shown in Table S1 and Fig. S1 and Fig. S2, which demonstrates the results utilizing different methods and area definitions.

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Table 1. Summary of reproductive traits of female skipjack tuna in the north western (>20° N) and central western (<20° N) Pacific Ocean of the western central Pacific Ocean (WCPO)

	North western Pacific (>20° N)	Central western Pacific (<20° N)
Spawning season	March–December	February–November*[1]
Minimum maturity size (FL cm)	38.2	40.0
Size at 50% maturity (FL cm)	63.0*[2]	49.6
Relative batch fecundity (oocytes/g)	89.0 ± 43.4*[3]	140.8 ± 58.3*[3]
Spawning interval (day)	1.97	1.95

*[1] except for August

*[2] excluded samples caught in May

*[3] mean ± standard deviation

Table 2. Summary of reproductive traits of female skipjack tuna in three areas of the western central Pacific Ocean (WCPO)

	Temperate (>30° N)	Subtropical (10° –30° N)	Tropical (<10° N)
Spawning season	June–September (Mainly July and August)	All year round	All year round
Minimum maturity size (FL cm)	40.9	38.2	40.0
Size at 50% maturity (FL cm)	55.9*1	53.7*2	50.1
Relative batch fecundity (oocytes/body weight (g))	83.6 ± 43.4*3	95.5 ± 49.9*3	169.6 ± 61.3*3
Spawning interval (day)	1.97	1.95	1.93

*1 restricted to samples caught in July and August

*2 excluded samples caught in May

*3 mean ± standard deviation

Table 3. Total number of females, matured female and ratio of matured female (%) in each region and length in 2cm intervals. Matured fish are based on histological observations. (a) No consideration of spawning season (included all month). (b) Consideration of spawning season (see **Table 2**).

(a)

FL (cm)	Temperate			Subtropical			Tropical			Overall		
	Total	Matured females	Ratio	Total	Matured females	Ratio	Total	Matured females	Ratio	Total	Matured females	Ratio
28	0	0	0.00	0	0	0.00	1	0	0.00	1	0	0.00
30	3	0	0.00	0	0	0.00	6	0	0.00	9	0	0.00
32	13	0	0.00	0	0	0.00	13	0	0.00	26	0	0.00
34	9	0	0.00	3	0	0.00	13	0	0.00	25	0	0.00
36	24	0	0.00	20	0	0.00	20	0	0.00	64	0	0.00
38	26	0	0.00	31	1	0.03	25	0	0.00	82	1	0.01
40	81	1	0.01	119	4	0.03	47	3	0.06	247	8	0.03
42	252	9	0.03	255	30	0.11	38	7	0.18	545	46	0.08
44	452	61	0.13	278	84	0.30	55	23	0.41	785	168	0.21
46	411	103	0.25	146	65	0.44	65	28	0.43	622	196	0.31
48	295	57	0.19	86	42	0.48	63	37	0.58	444	136	0.30
50	197	44	0.22	54	39	0.72	72	48	0.66	323	131	0.40
52	184	26	0.14	47	39	0.82	62	37	0.59	293	102	0.34
54	128	19	0.14	84	59	0.70	56	34	0.60	268	112	0.41
56	60	15	0.25	71	38	0.53	40	26	0.65	171	79	0.46
58	36	5	0.13	65	16	0.24	37	27	0.72	138	48	0.34
60	26	6	0.23	48	34	0.70	36	30	0.83	110	70	0.63
62	6	3	0.50	54	46	0.85	62	48	0.74	122	97	0.79
64	10	2	0.20	58	42	0.72	65	57	0.87	133	101	0.75
66	6	1	0.16	55	34	0.61	45	41	0.91	106	76	0.71
68	9	7	0.77	51	34	0.66	29	26	0.89	89	67	0.75
70	0	0	0.00	32	29	0.90	12	12	1.00	44	41	0.93
72	0	0	0.00	24	19	0.79	5	5	1.00	29	24	0.82
74	0	0	0.00	13	11	0.84	2	2	1.00	15	13	0.86
76	0	0	0.00	7	6	0.85	0	0	0.00	7	6	0.85
78	0	0	0.00	3	3	1.00	0	0	0.00	3	3	1.00
80	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00

Table 3. (continued).

(b)

FL (cm)	Temperate			Subtropical			Tropical			Overall		
	Total	Matured females	Ratio	Total	Matured females	Ratio	Total	Matured females	Ratio	Total	Matured females	Ratio
28	0	0	0.00	0	0	0.00	1	0	0.00	1	0	0.00
30	0	0	0.00	0	0	0.00	6	0	0.00	6	0	0.00
32	5	0	0.00	0	0	0.00	13	0	0.00	18	0	0.00
34	5	0	0.00	3	0	0.00	13	0	0.00	21	0	0.00
36	15	0	0.00	20	0	0.00	20	0	0.00	55	0	0.00
38	17	0	0.00	31	1	0.03	25	0	0.00	73	1	0.01
40	20	0	0.00	113	4	0.03	47	3	0.06	180	7	0.03
42	43	9	0.20	225	30	0.13	38	7	0.18	306	46	0.15
44	98	58	0.59	242	83	0.34	55	23	0.41	395	164	0.41
46	129	87	0.67	142	64	0.45	65	28	0.43	336	179	0.53
48	109	39	0.35	86	42	0.48	63	37	0.58	258	118	0.45
50	94	38	0.40	54	39	0.72	72	48	0.66	220	125	0.56
52	59	20	0.33	47	39	0.82	62	37	0.59	168	96	0.57
54	47	16	0.34	84	59	0.70	56	34	0.60	187	109	0.58
56	25	13	0.52	71	38	0.53	40	26	0.65	136	77	0.56
58	13	4	0.30	65	16	0.24	37	27	0.72	115	47	0.40
60	2	1	0.50	46	32	0.69	36	30	0.83	84	63	0.75
62	1	1	1.00	53	45	0.84	62	48	0.74	116	94	0.81
64	1	0	0.00	57	41	0.71	65	57	0.87	123	98	0.79
66	0	0	0.00	55	34	0.61	45	41	0.91	100	75	0.75
68	1	1	1.00	51	34	0.66	29	26	0.89	81	61	0.75
70	0	0	0.00	32	29	0.90	12	12	1.00	44	41	0.93
72	0	0	0.00	24	19	0.79	5	5	1.00	29	24	0.82
74	0	0	0.00	12	10	0.83	2	2	1.00	14	12	0.85
76	0	0	0.00	7	6	0.85	0	0	0.00	7	6	0.85
78	0	0	0.00	3	3	1.00	0	0	0.00	3	3	1.00
80	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00

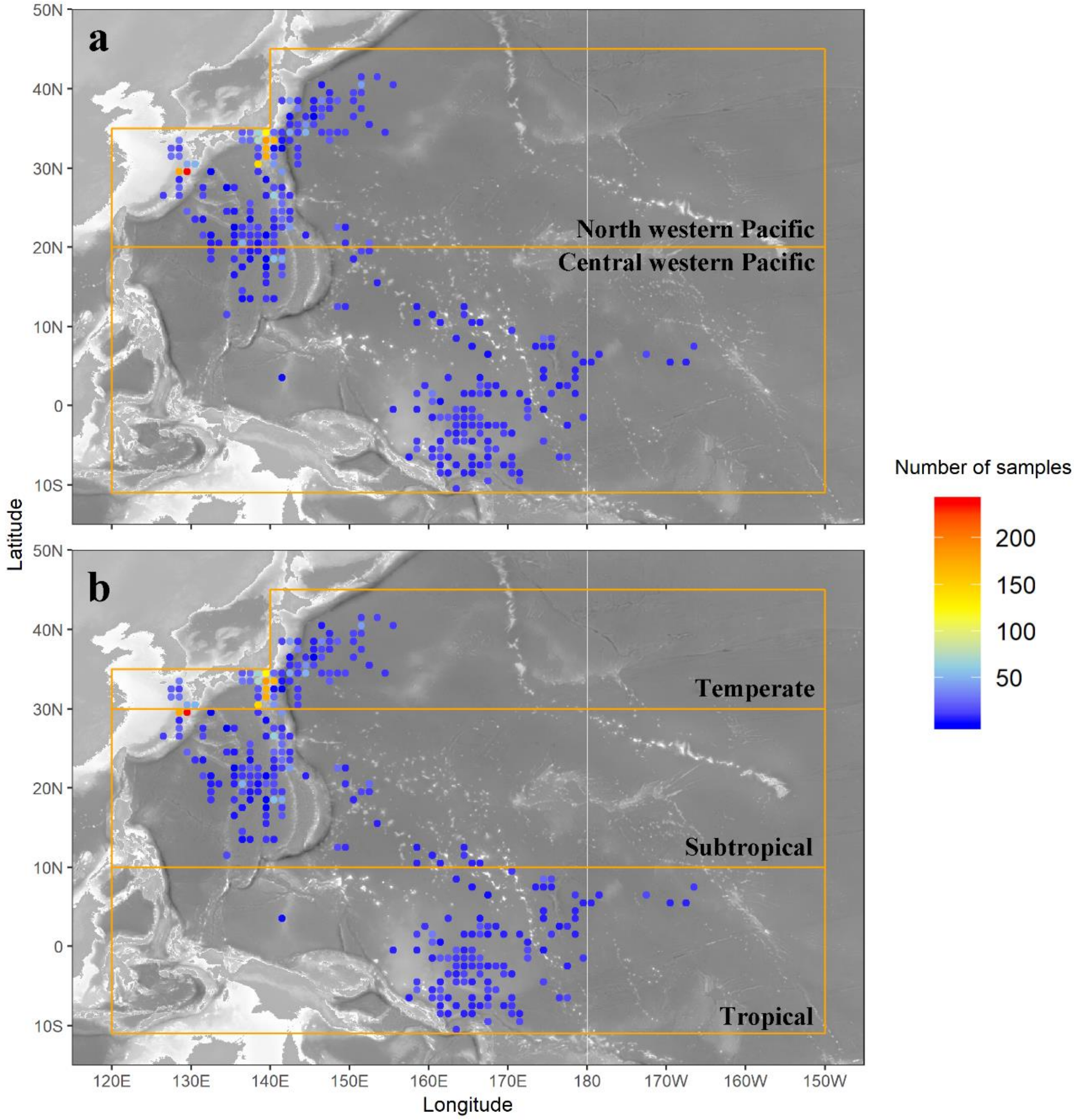

Figure 1. Area definitions and sampling locality of female skipjack tuna utilized to analyze reproductive traits except for sex ratio. The study area was divided into two areas on the 20° N line in a, and it was divided into three areas on the 30° N and 10° N lines in b.

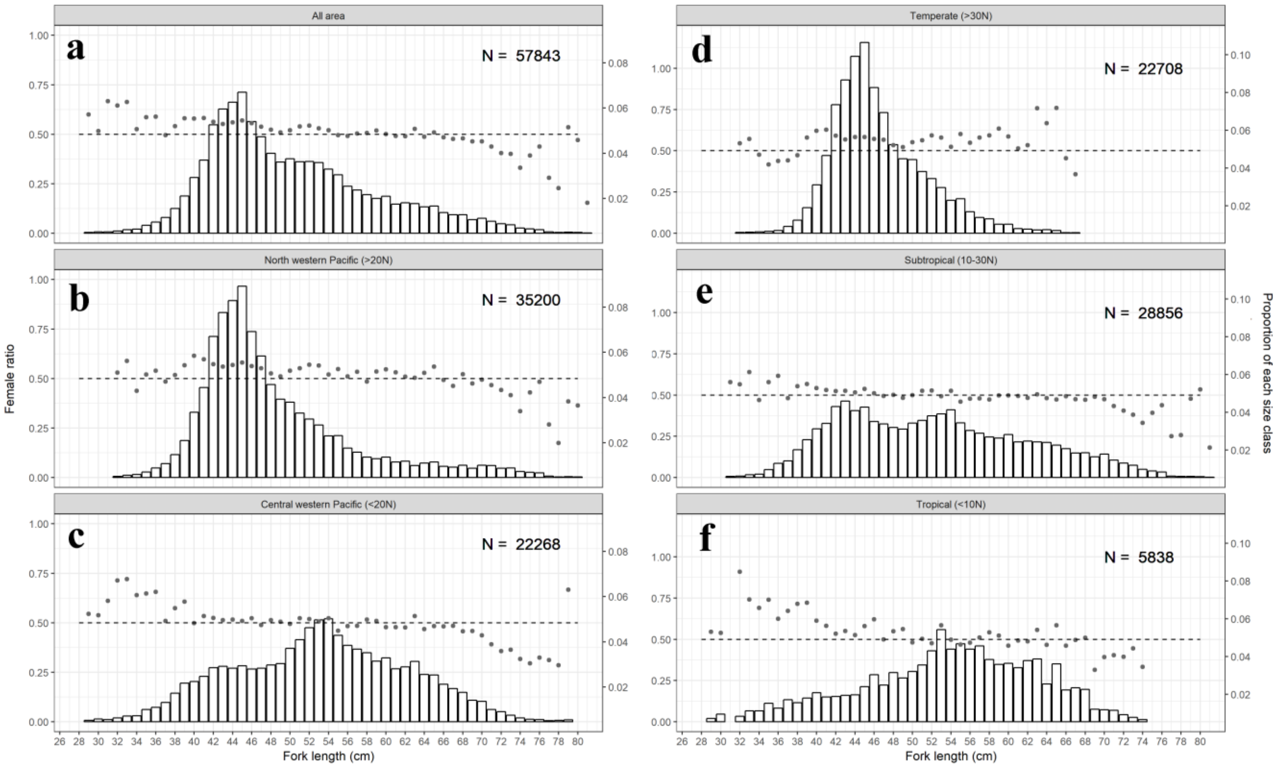

Figure 2. Female ratio of skipjack tuna in the whole WCPO and the divided areas, which was calculated from samples caught by Japanese research or training vessels from 1964 to 2017. Grey points and white bars indicate female ratio (left axis) and proportion of samples (right axis) in each size class. Size classes less than 10 samples were excluded. Dotted lines indicate 50% of the sex ratio.

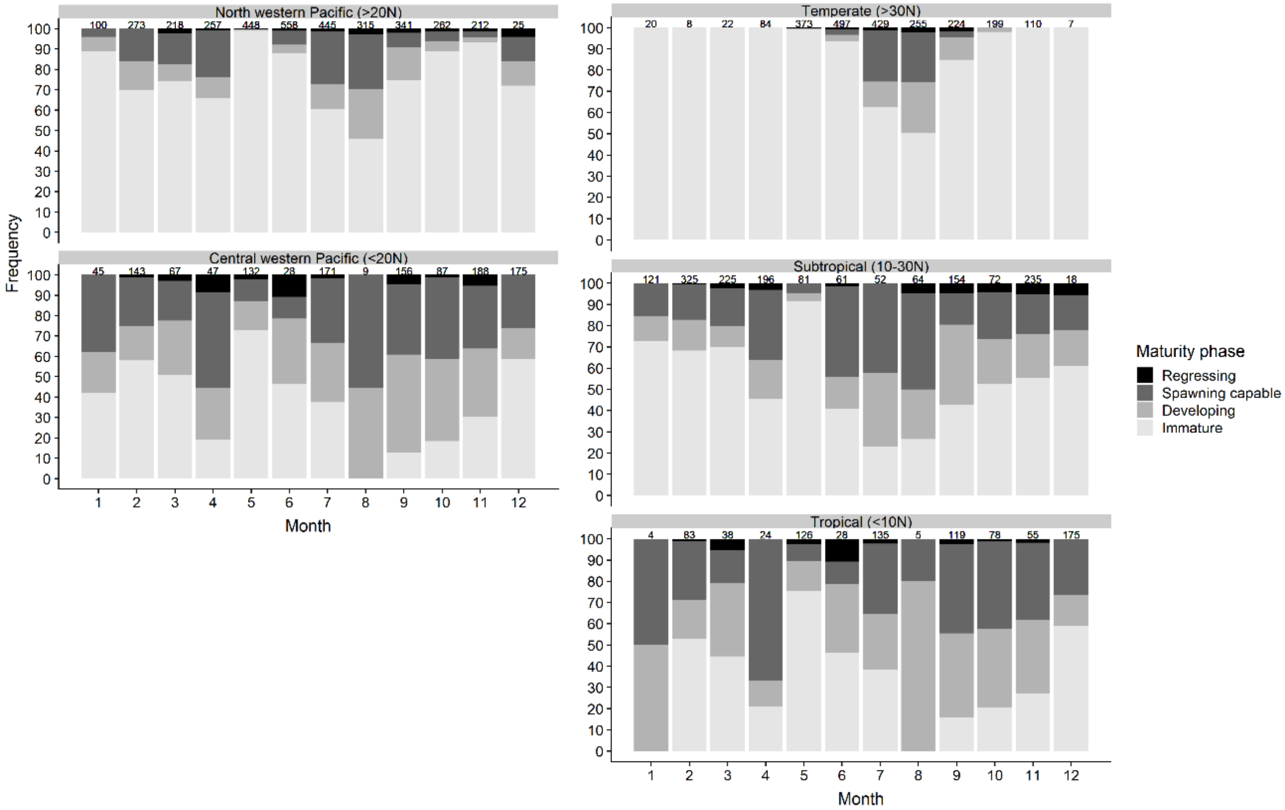

Figure 3. Monthly composition of maturity phases of female skipjack tuna in each defined area of the WCPO. The number above each bar represents the number of samples.

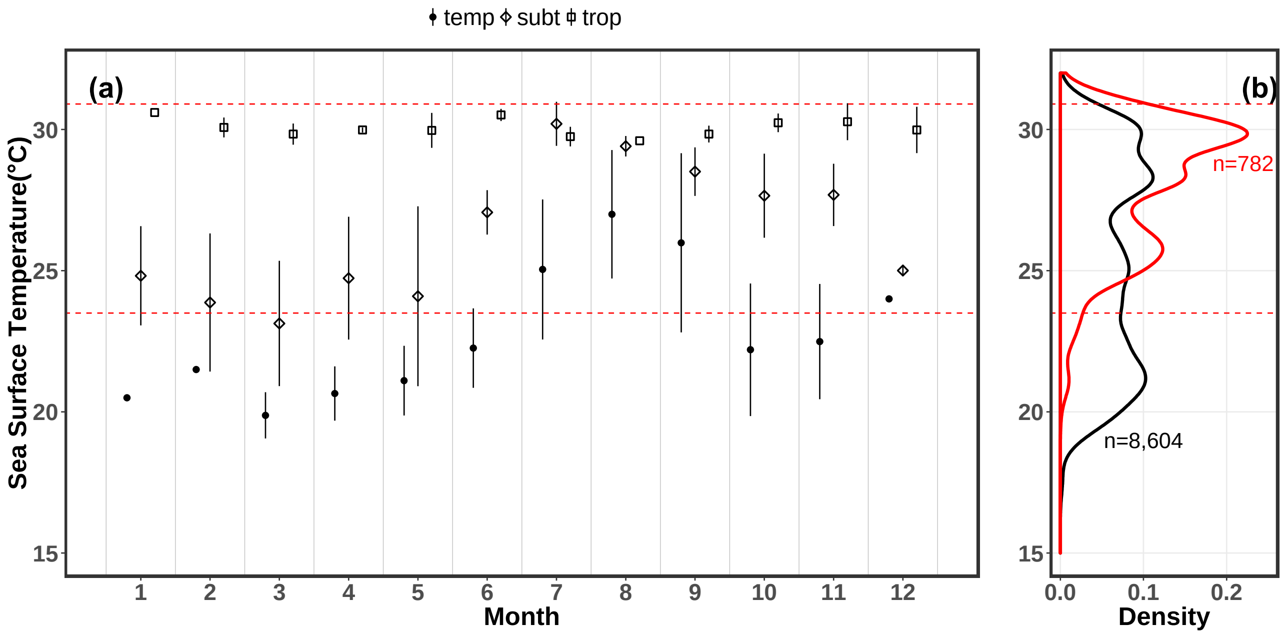

Figure 4. (a) Monthly averaged sea surface temperature (SST, °C) in each area in Fig. 1b, where skipjack were sampled. (b) Overall SST in the area where spawning capable female skipjack occurred (red) and all fish sampled (gray). Dashed red lines represent 5% (23.5°C) and 95% (30.5°C) of SST in the area where matured female skipjack occurred.

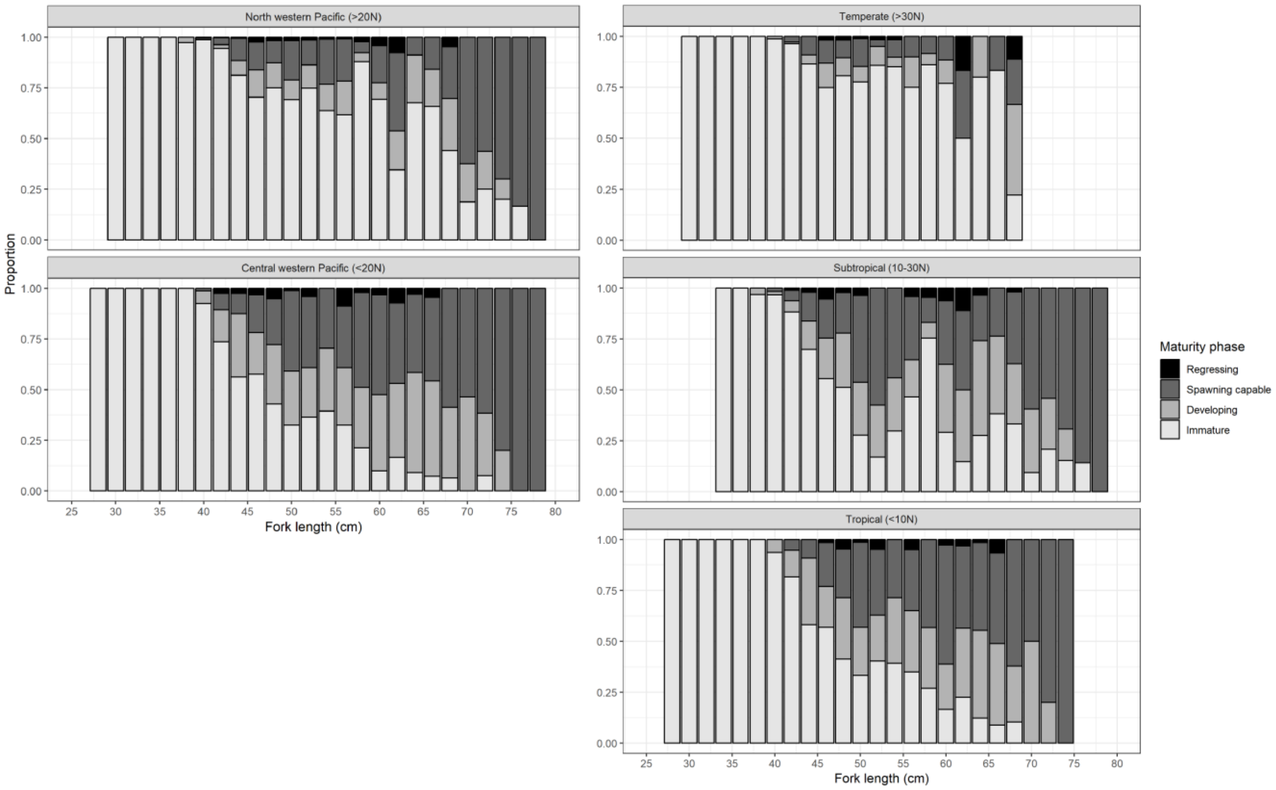

Figure 5. Proportion of ovarian maturity phases in each length class (FL in 2 cm intervals) of female skipjack tuna caught in each defined area.

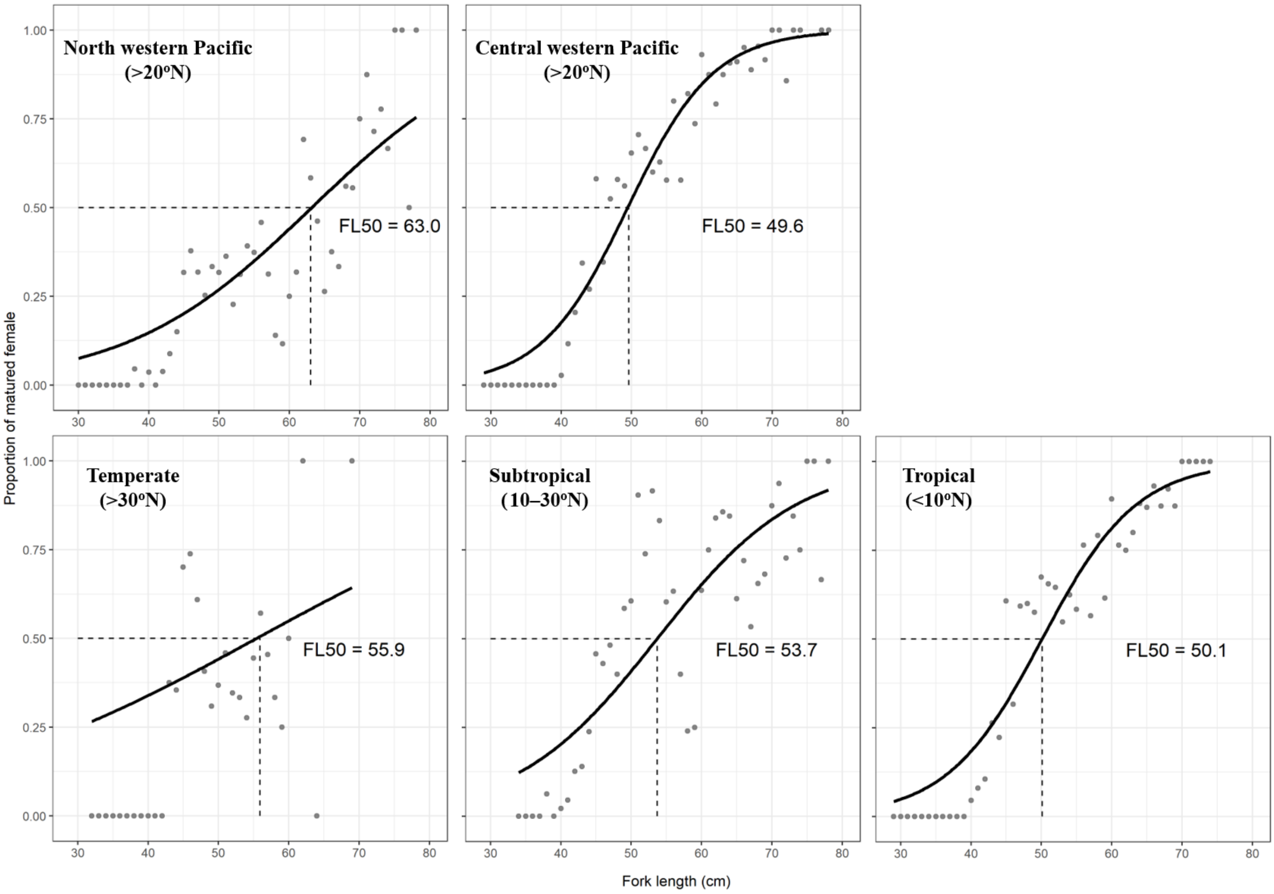

Figure 6. Relationships between the proportion of mature females and fork length observed in each area. In the north western Pacific area (>20° N), samples caught in May were excluded to remove the sampling bias, and in the temperate area (>30° N), samples of only July or August were adopted for the analysis considering the spawning season in the area.

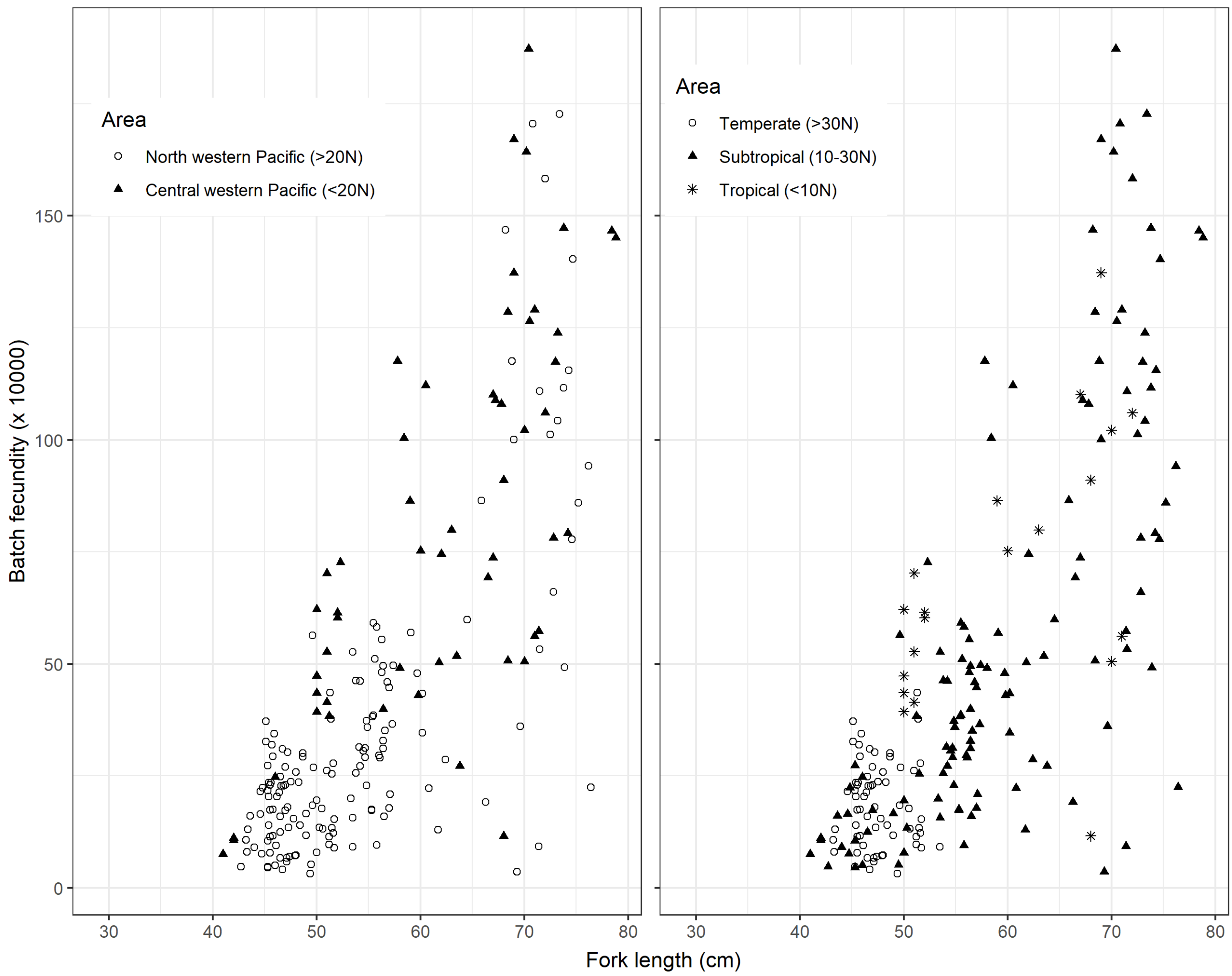

Figure 7. Relationships between batch fecundity and fork length in each defined area for 208 samples of female skipjack tuna.

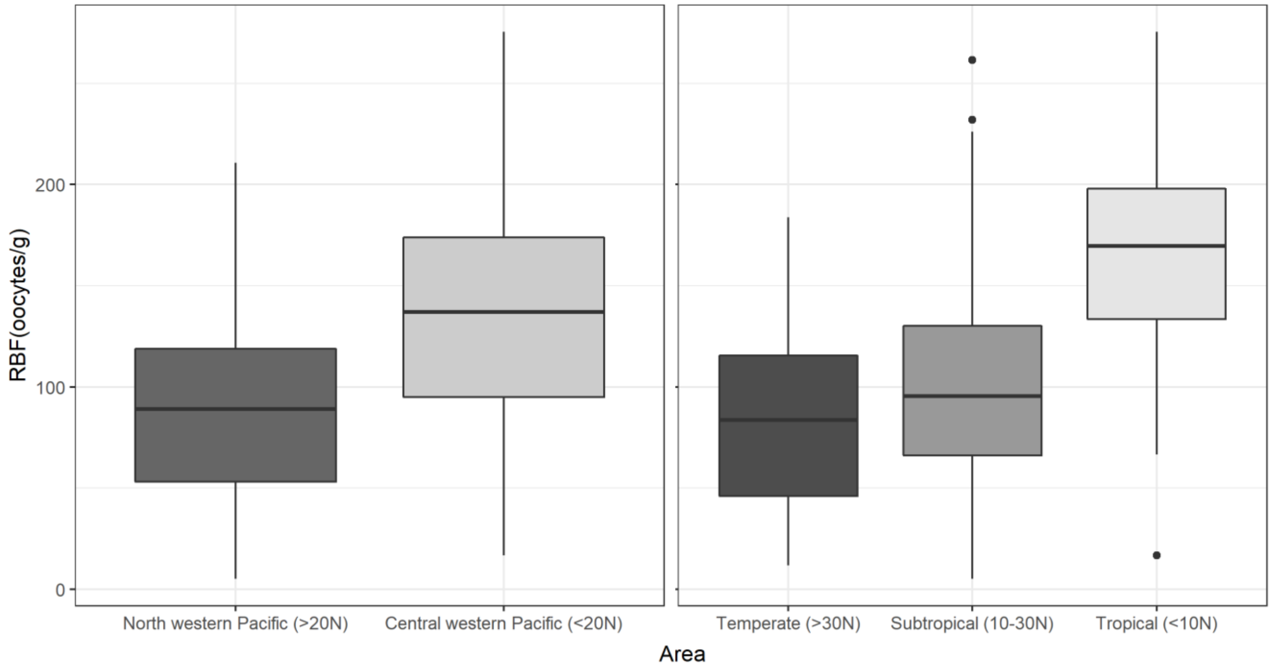

Figure 8. Distribution of relative batch fecundity in each area for 208 samples of female skipjack tuna.

Table S1. Summary of parameter estimates of three methods and estimated FL_{50}.

	No consideration of spawning season (all data included)	**Consideration of spawning season (included July and August in area of temperate, and excluded May in area of subtropical)**
Smoothing spline **R-package:** smooth.spline (stats) gam (mgcv)	**Smoothing parameter:** spar=0.608 (fixed) lambda = 0.00015 (estimated) FL_{50} = 58.9 cm	**Smoothing parameter:** spar=0.542 (fixed) lambda = 5.09e^{-5} (estimated) FL_{50} = 47.0 cm
Logistic regression **R-package:** glm **Equation:** $\boldsymbol{ln\left(\frac{P}{1-P}\right) = a + b * FL}$	**Estimated parameters:** a=-7.07 b=0.125 FL_{50} = 56.3 cm	**Estimated parameters:** a=-5.6, b=0.108 FL_{50} = 51.8 cm
Nonlinear regression **R-package:** minpack.lm **Equation:** $\boldsymbol{P = \frac{a}{1 + b * e^{FL}}}$	**Estimated parameters:** a=0.951, b=47.1, c=-0.13 FL_{50} = 56.8 cm	**Estimated parameters:** a=0.838, b=36.1, c=-0.17 FL_{50} = 51.0 cm

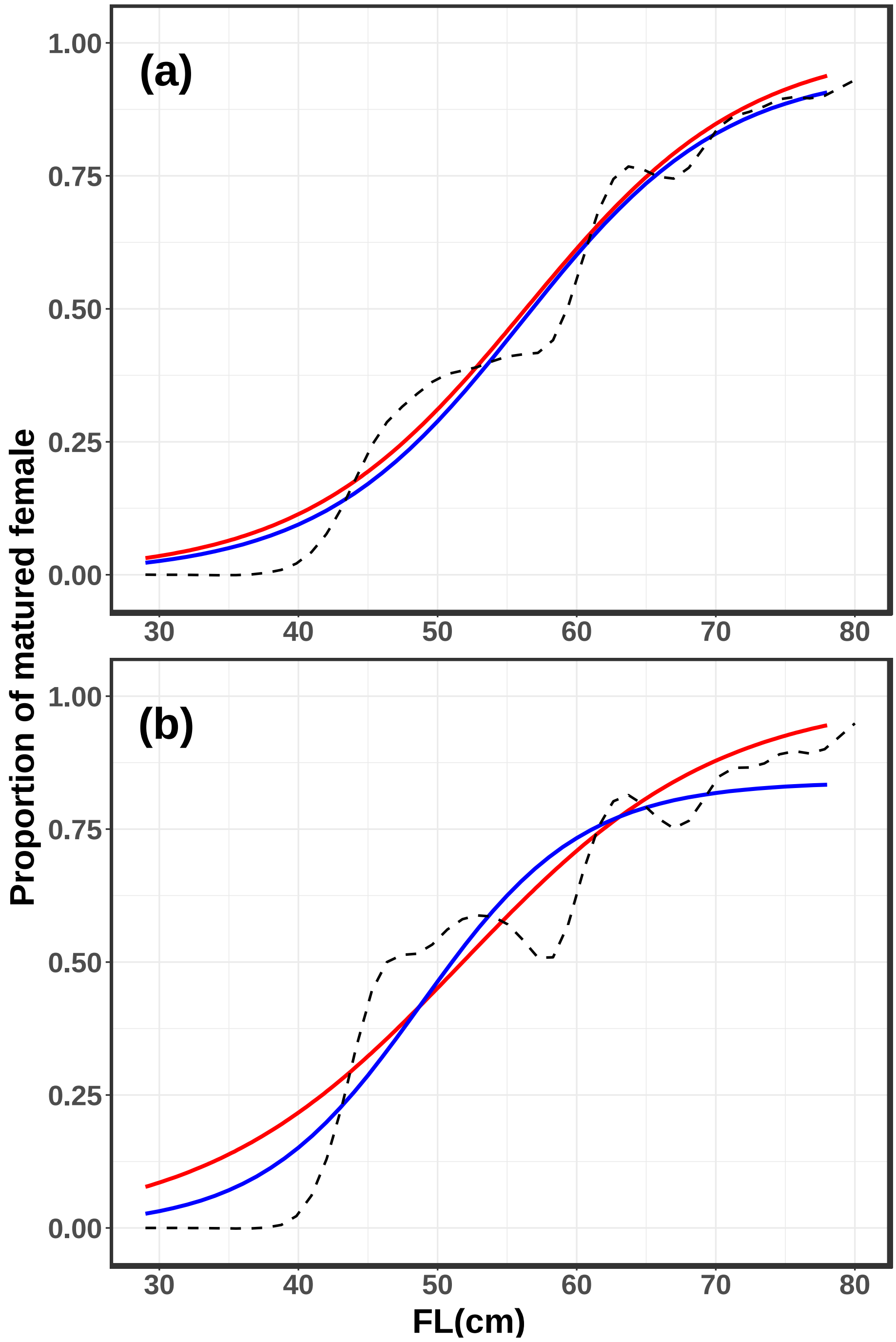

Figure S1. Relationships between proportion of matured female and each FL estimated by three different methods (black dashed: spline smooth function, red: logistic function, blue: nonlinear least square method). (a) No consideration of spawning season (all data included). (b) Consideration of spawning season (see Table S1).

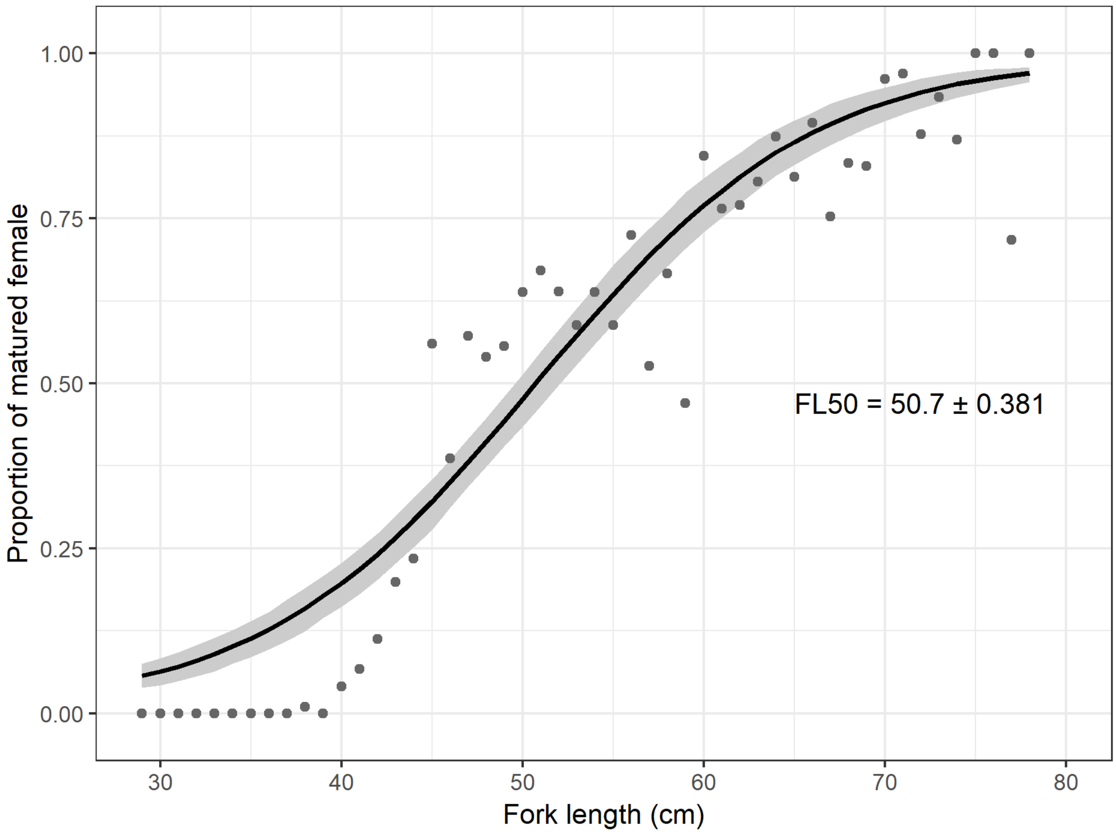

Figure S2. Relationships between proportion of matured female and each FL estimated by 100 times random sampling data based on a weighting of 5% temperate, 27% subtropical and 68% tropical. Black line and grey area are showing mean and range of estimated values by the generalized linear model (GLM) with logit function. Grey points indicate mean values of proportion of matured female in each FL.