Connectivity in juvenile yellowfin tuna

Online supplementary materials

Exploratory analysis

S1. Standard length

In small juveniles, $\delta^{18}O_{otolith}$ of the smallest (<5 cm SL) group in each area was significantly smaller than that of the larger group (TEM [$F_{1, 34} = 13.9$, $p < 0.001$]) and TROP [$F_{1, 137} = 24.6$, $p < 0.001$]) (**Fig. S**1). Significantly positive linear relationships between SL and $\delta^{18}O_{otolith}$ were found in the areas. No significant relationship was found between small juvenile $\delta^{13}C_{otolith}$ and the smallest (<5 cm SL) group (TEM; $F_{1, 34} = 0.01$, $p = 0.943$, TROP; $F_{1, 137} = 3.17$, $p = 0.077$). Significant linear relationships were not found between SL and $\delta^{13}C_{otolith}$ (TEM; $p = 0.162$, TROP; $p = 0.159$). $\delta^{18}O_{otolith}$ of small juveniles in TEM was larger than that in TROP in every length group (categorized by 5 cm SL interval), and a significant difference in small juvenile $\delta^{18}O_{otolith}$ was found between the two areas ($F_{1, 49} = 10.15$, $p = 0.002$) in 2012. In GLM analysis the effect of the length group was considered as a covariate, and its effect was significant ($F_{3, 49} = 8.82$, $p < 0.001$). In 2012, small juvenile $\delta^{13}C_{otolith}$ in TEM was smaller than that in TROP in every length group, and a significant difference in $\delta^{13}C_{otolith}$ of small juveniles between the areas was found ($F_{1, 49} = 33.91$, $p < 0.001$). In GLM analysis, the effect of the length group as a covariate was significant ($F_{3, 49} = 1.75$, $p = 0.169$).

In large juveniles, $\delta^{13}C_{otolith}$ of the largest group (>40 cm SL) was significantly larger than that of the smaller size group in the areas (TEM [$F_{1, 249} = 4.5$, $p = 0.035$] and TROP [$F_{1, 275} = 6.33$, $p = 0.012$]) (**Fig. S**2). A significantly positive linear relationship was found between SL and $\delta^{13}C_{otolith}$ in TEM ($p < 0.001$) whereas this relationship was not significant in TROP ($p = 0.273$). $\delta^{18}O_{otolith}$ of the largest group was significantly larger than that of the smaller group in the areas (TEM [$F_{1, 249} = 19.6$, $p < 0.001$]) and TROP [$F_{1, 275} = 9.32$, $p = 0.002$]). No significant linear relationships was found between SL and $\delta^{18}O_{otolith}$ in TEM ($p = 0.061$), whereas this relationship was significant in TROP ($p = 0.009$). No significant difference in $\delta^{13}C_{otolith}$ of large juveniles was found between the two areas ($F_{1, 519} = 0.62$, $p = 0.429$). In GLM analysis, the effect of the length group as a covariate was significant ($F_{7, 519} = 4.83$, $p < 0.001$). $\delta^{13}C_{otolith}$ of large juveniles in TROP was significantly larger than that in TEM ($F_{1, 519} = 67.6$, $p < 0.001$). The length group as a covariate was significant ($F_{7, 519} = 5.31$, $p < 0.001$). The largest group from TEM was collected from one sampling station (35°N, 146°E) by a commercial pole-and-line fishery in 2013, and a large juvenile group from TROP was only collected from 2011. Thus, the effect of SL on large juveniles, sampling year, and sampling location may not be separated. SL (<5 cm SL and >40 cm SL) could have an effect on stable isotope ratios , which can be addressed in sensitivity analysis limiting the SL range of specimens (e.g., 5–40 cm SL) to investigate its effect on classification analysis.

S2. Year sampled

Comparison of the stable isotopes of small juveniles among the year sampled showed that a significant difference was only found for $\delta^{13}C_{otolith}$ in TROP ($F_{1, 134} = 4.96$, $p < 0.001$; **Fig. S**3). No significant differences of $\delta^{13}C_{otolith}$ of large juveniles were observed in TEM among the year sampled, whereas a significant difference in $\delta^{13}C_{otolith}$ of large juveniles was found in TROP ($F_{1, 273} = 12.85$, $p < 0.001$). The annual changes in $\delta^{18}O_{otolith}$ were similar in the two areas, and a wider distribution of $\delta^{18}O_{otolith}$ in 2013 than in other years was also found in the two areas. A significant difference in $\delta^{18}O_{otolith}$ among the years sampled was found in TEM ($F_{3, 247} = 6.82$, $p < 0.001$). Multiple comparison showed that the $\delta^{18}O_{otolith}$ of large juveniles in TEM in 2013 was significantly lower than that in other years (2011: $p = 0.008$; 2014: $p = 0.001$; 2015: $p = 0.020$). In TROP, $\delta^{18}O_{otolith}$ also varied significantly among the years sampled ($F_{3, 273} = 22.33$, $p < 0.001$), except for a comparison between 2014 and 2015 ($p = 0.425$). The stable isotopes of small juveniles did not vary among the years sampled compared with those of large juveniles. Therefore, a similarity in environmental conditions of the spawning and nursery grounds of small juveniles was observed in TEM, although no small juvenile specimens were collected during the 2014 and 2015 El Niño.

S3. Longitude sampled within each area

The effect of longitude (grouped by 10° interval in longitude) on the stable isotopes per year was not clear because of its narrow range for small juveniles (**Fig. S**4). Significantly negative linear relationships between longitude (from west to east) and stable isotopes in TROP ($\delta^{13}C_{otolith}$ [$p < 0.001$]) and $\delta^{18}O_{otolith}$ [$p = 0.011$]) were found. In TEM, the relationship was inverse (positive); however, it was derived from one lower value for the stable isotopes in the sampling location of 125°E degree. A significantly increasing trend was observed from west to east in the areas for the large juveniles ($\delta^{13}C_{otolith}$ in TROP, $p < 0.001$; $\delta^{18}O_{otolith}$ in TROP, $p = 0.015$; $\delta^{18}O_{otolith}$ in TEM, $p < 0.001$; **Fig. S**5).

S4. Latitude sampled within each area

Significantly positive linear relationships were found between the latitude and stable isotopes in TROP for small juveniles ($\delta^{13}C_{otolith}$ [$p < 0.001$]) and $\delta^{18}O_{otolith}$ [$p = 0.017$]; **Fig. S**6). In TEM, the relationship was also positive; however, it was derived from one lower value for stable isotopes in the sampling location of 26°N. $\delta^{18}O_{otolith}$ stable isotopes in TEM for large juveniles had a significantly increasing trend ($p < 0.001$; **Fig. S**7).

S5. Least square means for covariates

The covariates SL and year sampled also had a significant effect (SL: $F_{2, 166} = 45.15$, $p < 0.001$; year: $F_{10, 334} = 2.18$, $p = 0.018$). Four factors, including the year, longitude, latitude, and SL, were tested to investigate the effect of each factor on stable isotopes of small juveniles. In TEM, SL was the only significant factor ($F_{2, 30} = 6.01$, $p = 0.006$) among the four factors. $\delta^{18}O_{otolith}$ of the smallest SL group was significantly lower than that of another size group ($p = 0.001$), whereas $\delta^{13}C_{otolith}$ was not significantly different between the size groups ($p = 0.585$). In addition, no significant differences in stable isotopes by longitude and latitude were observed in TEM (longitude: $F_{2, 30} = 2.34$, $p = 0.114$; latitude: $F_{2, 30} = 0.58$, $p = 0.563$). In TROP, the significant influence of three factors (year, latitude, and SL) on the stable isotopes was investigated. In addition, the longitude was not addressed in this analysis because of a missing value for post-hoc test. A significant difference in stable isotopes by latitude was also found ($F_{6, 260} = 9.20$, $p < 0.001$). The least square mean of $\delta^{13}C_{otolith}$ of 7.5°N (10°N to 5°N; −9.32) were higher than those of the other three groups (2.5°N: −9.84; 2.5°S: −9.80; 7.5°S: −9.71). The $\delta^{18}O_{otolith}$ of 7.5 °N (−2.45) was also higher than that of the other three groups (2.5 °N: −2.63; 2.5 °S: −2.60; 7.5 °S: −2.92). The covariates SL and year sampled also had a significant effect (SL: $F_{2, 521} = 24.42$, $p < 0.001$; year: $F_{6, 1044} = 26.43$, $p < 0.001$).

In large juveniles, the effects of the other factors were tested. In TEM, the sampling position was grouped into two fishing grounds, namely, the southeast and northwest of Japan. The sampling year and fishing grounds were evaluated (year sampled: $F_{6, 492} = 11.74$, $p < 0.001$; fishing ground: $F_{2, 245} = 34.97$, $p < 0.001$). $\delta^{13}C_{otolith}$ (2011: −9.84 ± 0.327; 2013: −10.2 ± 0.0758; 2014: −10.3 ± 0.0513; 2015: −10.2 ± 0.0532) of large juveniles from TEM did not show significant annual differences, whereas $\delta^{18}O_{otolith}$ (2011: −1.19 ± 0.0593; 2013: −2.75 ± 0.0982; 2014: −2.49 ± 0.0314; 2015: −2.54 ± 0.0311) presented a significantly lower value in 2013 than in other years ($p < 0.001$). No significant difference in $\delta^{13}C_{otolith}$ was observed between the fishing grounds, whereas $\delta^{18}O_{otolith}$ from the northwest fishing ground was significantly higher than that from the southwest one. However, the effect of the sampling years and fishing grounds could not be separated because of the confounding effect of these factors. In TROP, all four factors showed a significant effect on the stable isotopes (MANOVA, year sampled: $F_{6, 528} = 23.72$, $p < 0.001$; latitude: $F_{10, 528} = 7.49$, $p < 0.001$; longitude: $F_{6, 528} = 2.20$, $p = 0.041$; SL: $F_{2, 263} = 3.60$, $p = 0.028$). $\delta^{13}C_{otolith}$ of large juveniles collected from TROP (2011: −9.69 ± 0.145; 2013: −9.41 ± 0.0705; 2014: −9.8 ± 0.0532; 2015: −10 ± 0.064) showed significant annual differences in some cases (2013 vs. 2014: $p = 0.015$; 2013 vs. 2015: $p < 0.001$). Significant annual differences in the $\delta^{18}O_{otolith}$ of large juveniles from TROP were also found, except for the years 2014 and 2015 ($p = 0.866$; 2011: −2.06 ± 0.0425; 2013: −2.95 ± 0.0647; 2014: −2.59 ± 0.0487; 2015: −2.5 ± 0.03). In this analysis, the least square mean of $\delta^{13}C_{otolith}$ by longitude showed an increasing trend from 147.5°E to 167.5°E (147.5°E: −9.51; 152.5°E: −9.42; 157.5°E: −9.40; 162.5°E: −9.16; 167.5°E: −9.04). A 0.0241‰ increase in $\delta^{13}C_{otolith}$ per 1° longitudinal changes from west to east was found if a value in 142.5 °E (−9.18) was neglected. This low value was obtained from only three data points in one sampling station. The least square mean of $\delta^{18}O_{otolith}$ of 142.5°E (−3.46) was significantly lower than that on other longitude groups (147.5°E: −2.64; 152.5°E: −2.64; 157.5°E: −2.51; 162.5°E: −2.19; 167.5°E: −2.37), which showed a 0.0201 ‰ increase in $\delta^{18}O_{otolith}$ per 1° longitudinal changes from west to east if the value in 142.5°E was neglected. $\delta^{13}C_{otolith}$ of large juveniles collected from TROP did not show significant differences by latitude. $\delta^{18}O_{otolith}$ presented a significant difference between 2.5°N and 7.5°S ($p = 0.023$). In small juveniles in TROP, there could be a decreasing trend from north to south (7.5°N: −2.49, 2.5°N: −2.59; 2.5°S: −2.62; 7.5°S: −2.83). $\delta^{13}C_{otolith}$ of large juveniles from TROP under the 40 cm SL was significantly lower than that of groups with a larger SL ($p = 0.007$), whereas $\delta^{18}O_{otolith}$ did not show significant differences by SL.

S6. Best classification model

In preliminary analysis including SVM with three kernel types (linear, quadratic, and radial basis), linear discriminant function analysis, quadratic discriminant function analysis, and logistic discriminant analysis, were also tested (Table S2, Fig. S8). These analyses were conducted using an e1071 library for SVMs, MASS library for linear and quadratic discriminant models, and stat library for logistic discriminant analysis. In addition, the variance-covariance matrix of the predictor variables was different among the five sampling units of small juveniles (BOX-M test, $P = 0.004$); therefore, the quadratic discriminant function analysis was better than the linear model.

The best classification success rate was 92.6% on the SVM-quadratic model when all stable isotope data for small juveniles in the two areas were combined; however, the success rate for TEM in the model was poor (66.7%). Higher rate were derived from a higher success rate (99.3%) in TROP. Thus, the result of GAM was selected because of its better performance with regard to the overall classification success rate (92.0%) and its better balance of the rates in TEM (72.6%) and TROP (97.1%), although the results of all classification analyses were close to one another.

Additional methodology texts

S7. Towing methodology for the mid-water trawl

Juvenile yellowfin tuna specimens were collected using a mid-water otter trawl of fishery training vessels (Oumi-maru from 2005 to 2007) and research vessels (Shunyo-maru from 2011 to 2013). From 2005 to 2007, an 89-m-long trawl net with a 20 m × 20 m mouth opening was used, of which the mesh size was 100 cm × 100 cm at the mouth, gradually decreasing to 8 mm × 8 mm at the cod end (revised TANSYU-type Nichimo Co. Ltd., Tanabe & Niu 1998). The trawl net was towed at 4–5 knots for 1 h at target depths of 0–200 m (10 layers with 6-min tow for each layer) and 80–120 m (two layers with 30-min tow). From 2011 to 2013, a 93-m-long trawl net with a 30 m × 30 m mouth opening was used, of which the mesh size was 100 cm × 100 cm at the mouth and 16 mm × 16 mm at the cod end (NBT-2P-SY type, Nitto Seimo Co., Ltd.).

S8. Conversion factors

The length of these collected small juveniles was expressed as SL before fixation by ethanol. The relationship between the SLs before and after fixation was expressed using the following equation and was used to correct the SL of fixed small juveniles (range: 3.0 to 12.3 cm SL):

$SL_{before} = 1.01* SL_{after} + 0.28$ ($R^2 = 0.98$, $P < 0.001$, n = 35),

where SL_{before} and SL_{after} were the SL of small juveniles before and after fixation, respectively.

For easy comparison with other studies, the conversion equation between the SL and FL for large juveniles by area wa presented (TROP: FL = 0.5747 + 1.015 * SL, $R^2 = 0.9975$, n = 303, range: 27.6−54.2 cm SL, TEM: FL = 0.303 + 1.025 * SL, $R^2 = 0.9991$, n = 500, range: 18.8−55.3 cm). In addition, specimens using the abovementioned equations included the samples outside of this study, but the sampling areas and sampling gears were the same in this study. Large fish were collected from commercial fishery products at fishery ports; their catch dates and positions were collected by interview surveys at the ports and a logbook collecting system (Purse seine: Yaizu in Shizuoka pref., Makurazaki and Yamagawa in Kagoshima pref.; Pole-and-line: Katsuura in Chiba pref.; Troll: Yonaguni in Okinawa pref.).

S9. Compilation of oceanographic conditions for large juveniles for each area

Four layers at a depth of 5 to 35 m for TEM and 10 layers at a depth of 35 m to 95 m for TROP in the database were compiled. The data in these layers were processed using year–month resolution and 1° × 1° in the two study areas (**Fig**. 1). Then, the temperature and salinity of the target depths were averaged.

S10. Definition of El Niño (La Niña) in this study

El Niño (La Niña) is defined as a period when the 5-month running mean SST deviation for the index NIÑO.3 (5°S–5°N, 90°–150°W) is 0.5°C (−0.5°C) or higher (lower) for six consecutive months or longer. During the study period, the three oceanographic conditions (neutral, El Niño, and La Niña) were determined for each month, starting from January 2005 to December 2015 (Table S1).

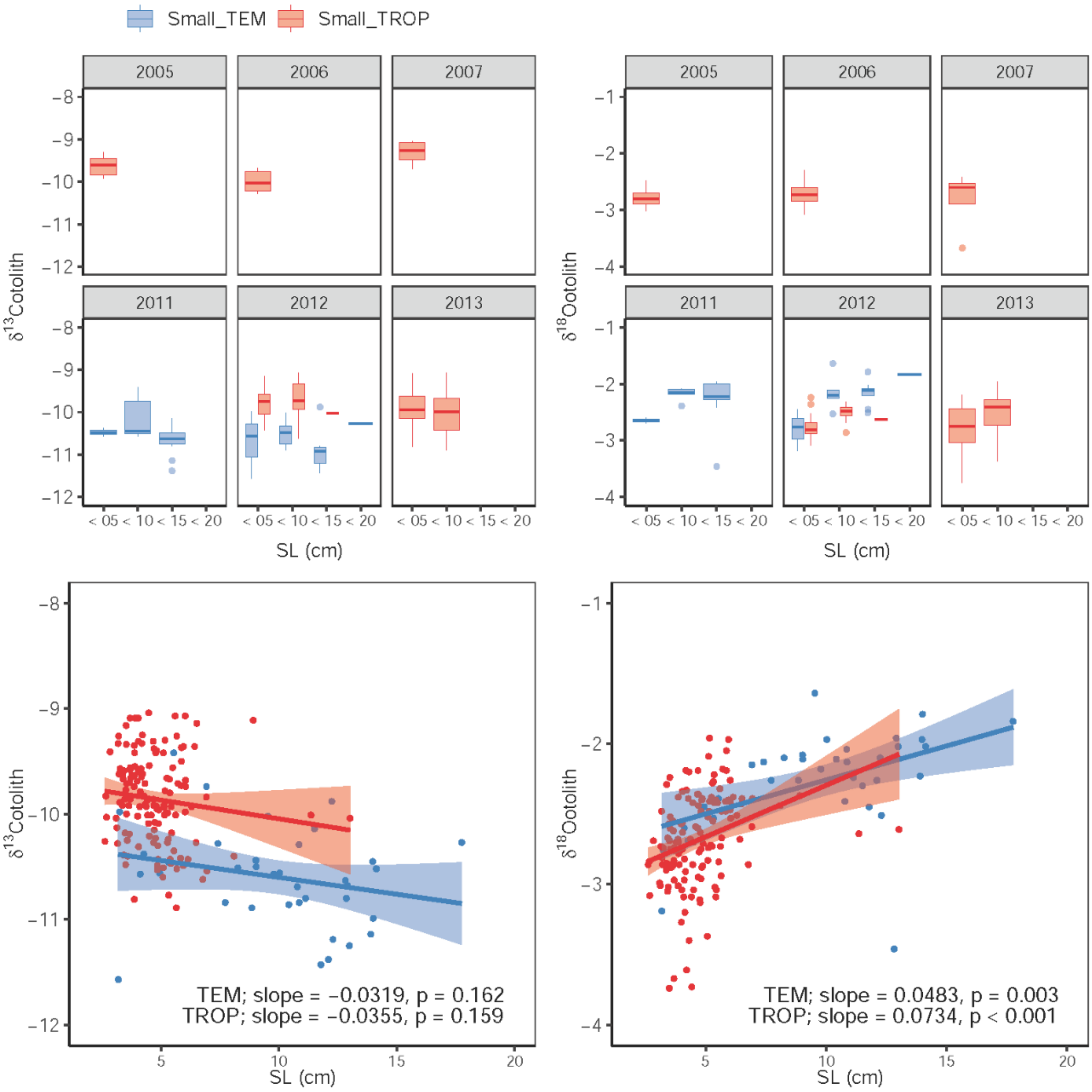

Fig. S1. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and standard length (SL [cm]) for small juveniles by temperate area (TEM) and tropical area (TROP) and by year (boxplots). The blue and red shade around regression lines show 95% confidence interval.

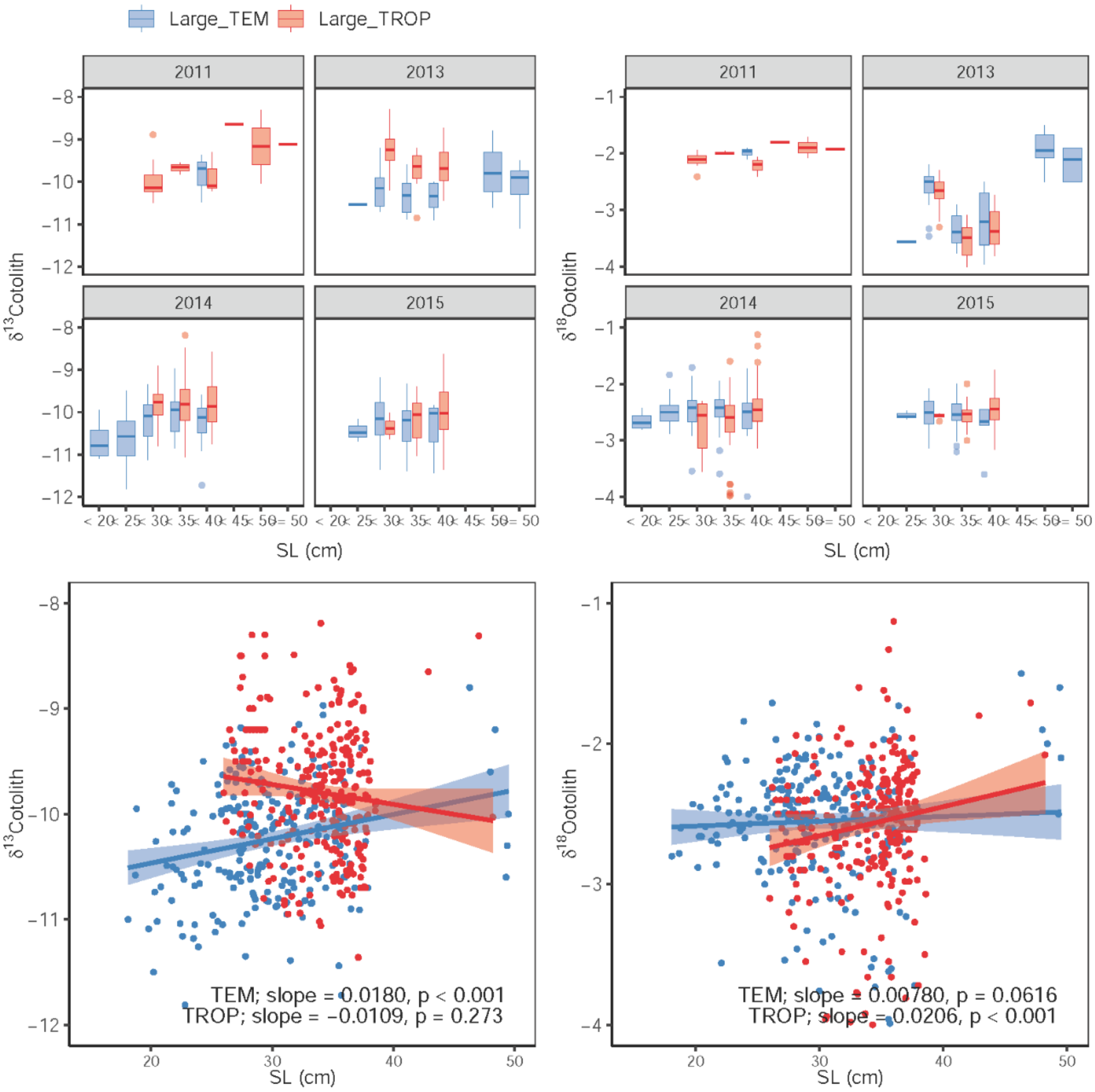

Fig. S2. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and standard length (SL [cm]) for large juveniles by temperate area (TEM) and tropical area (TROP) and by year (boxplots). The blue and red shade is as in Fig. S1.

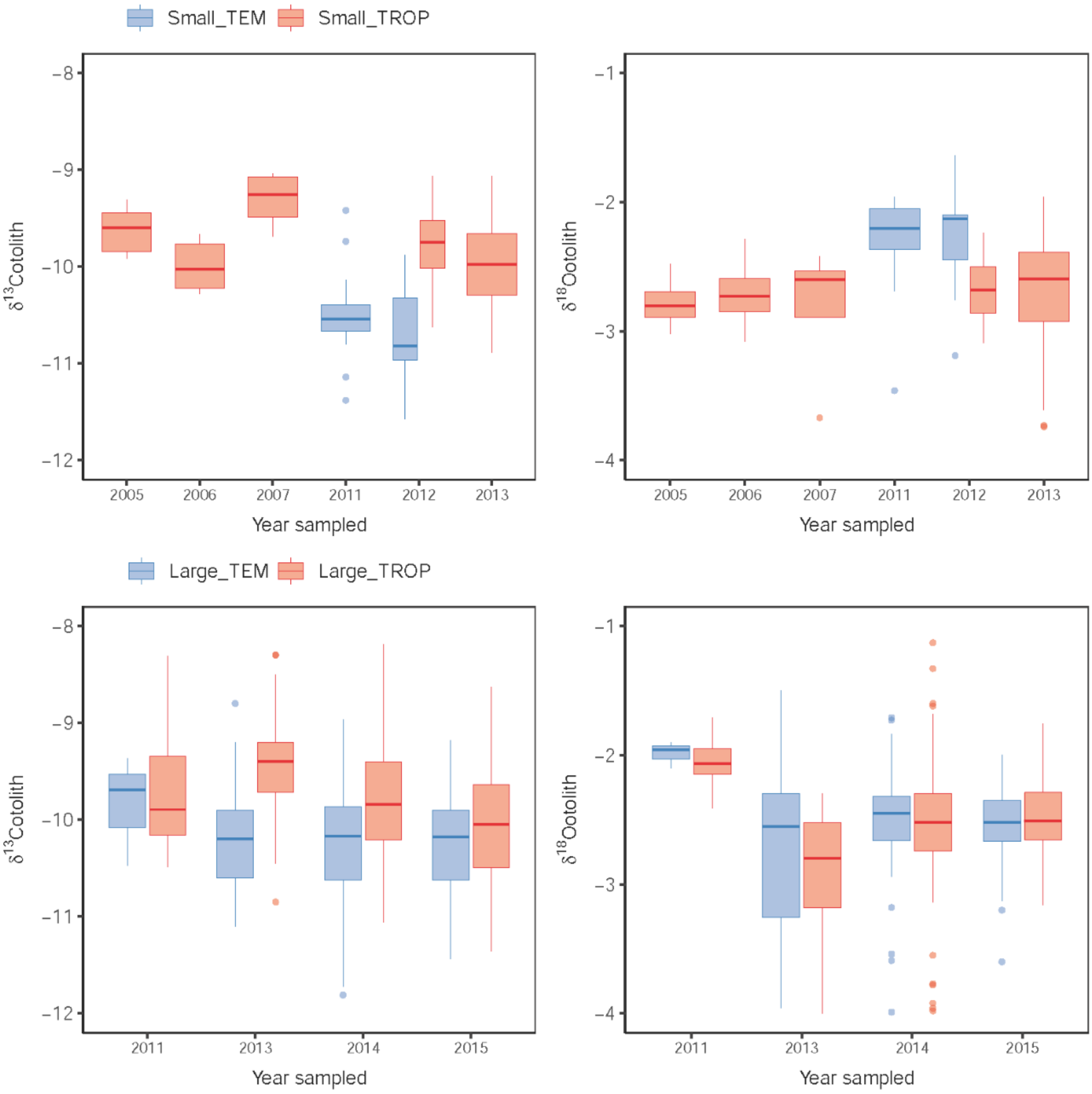

Fig. S3. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and years sampled for small and large juveniles by temperate area (TEM) and tropical area (TROP) and by year.

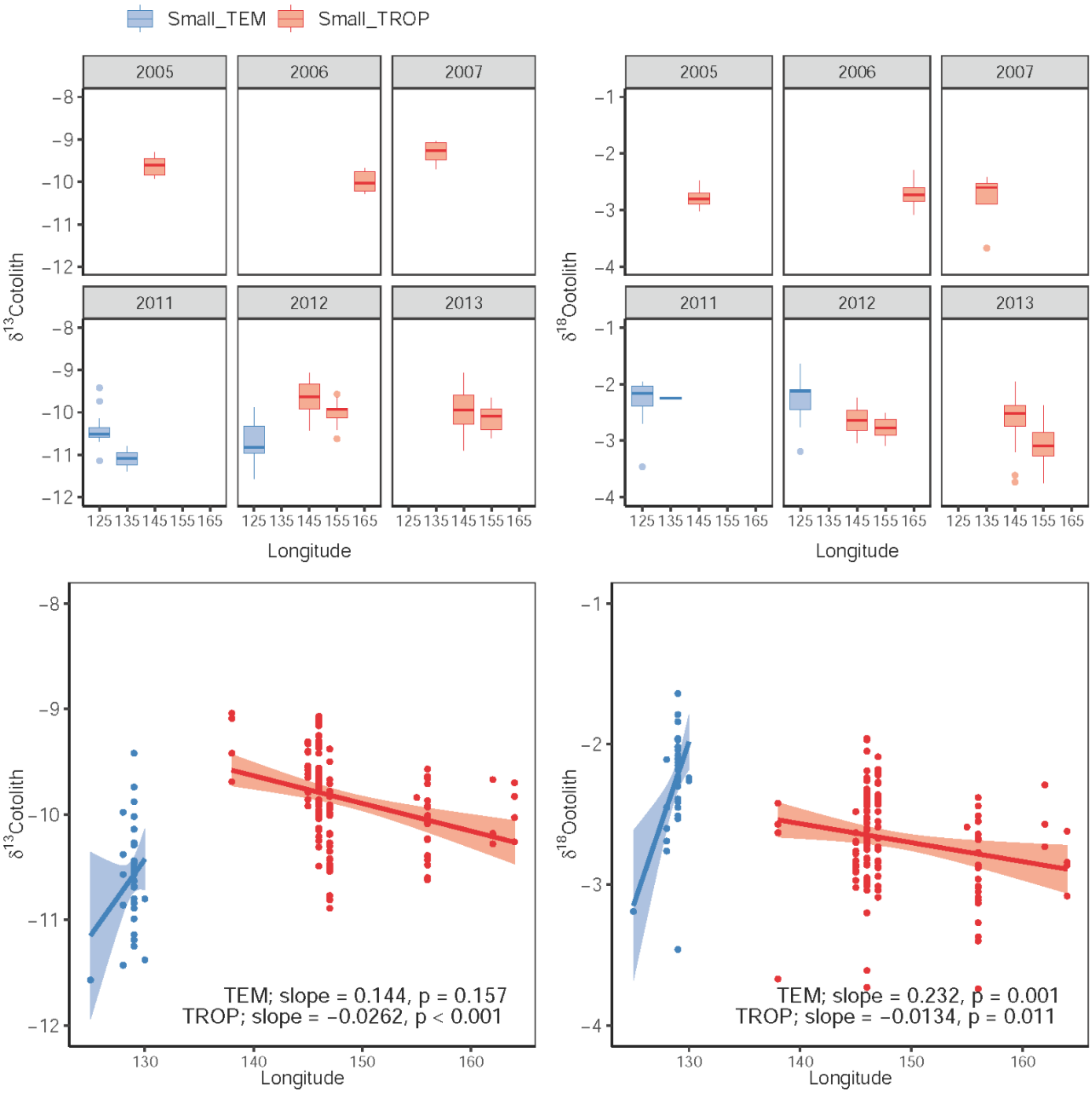

Fig. S4. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and longitude for small juveniles by temperate area (TEM) and tropical area (TROP). The blue and red shade is as in Fig. S1.

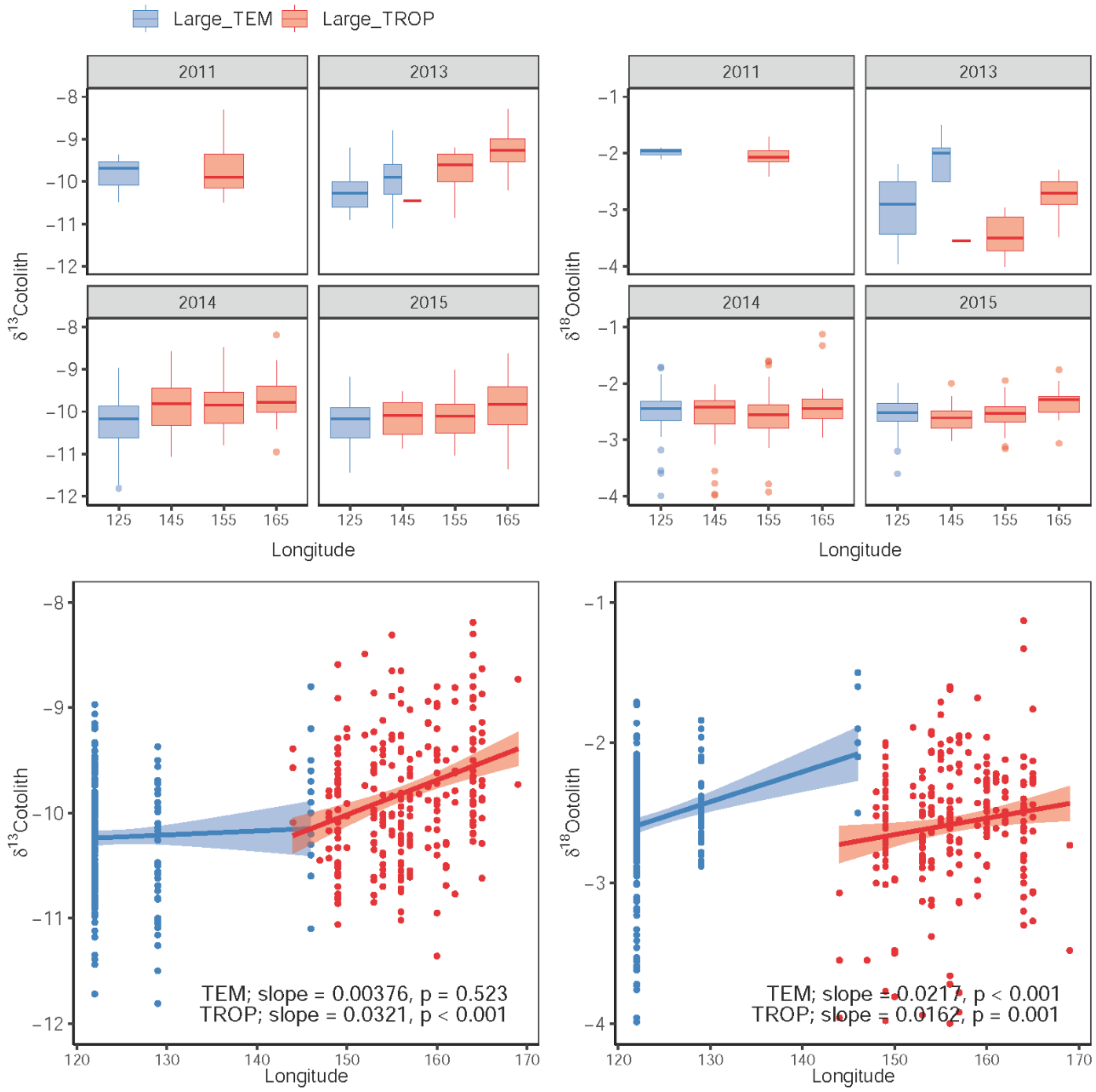

Fig. S5. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and longitude for large juveniles by temperate area (TEM) and tropical area (TROP). The blue and red shade is as in Fig. S1.

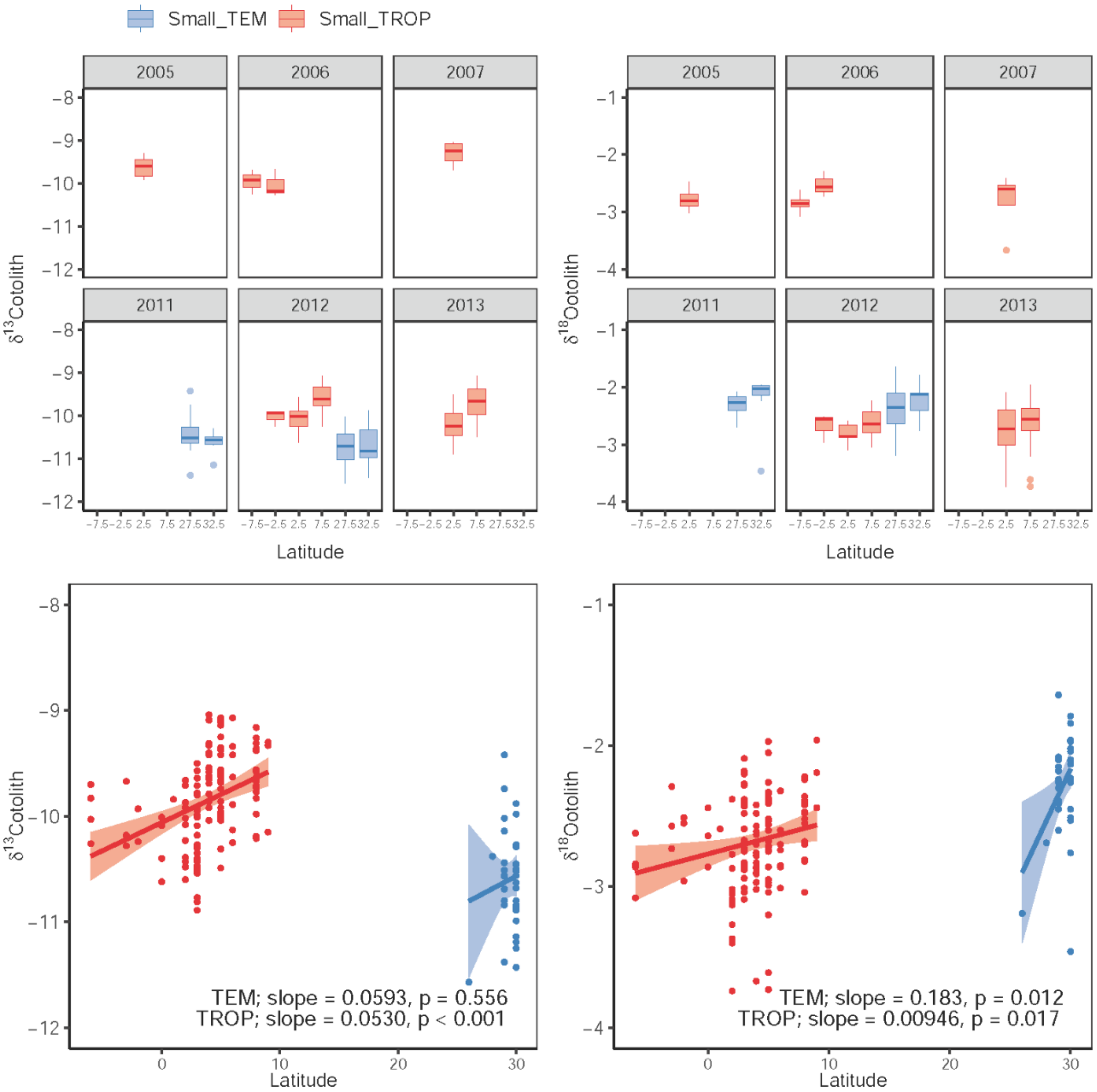

Fig. S6. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and latitude for small juveniles by temperate area (TEM) and tropical area (TROP). The blue and red shade is as in Fig. S1.

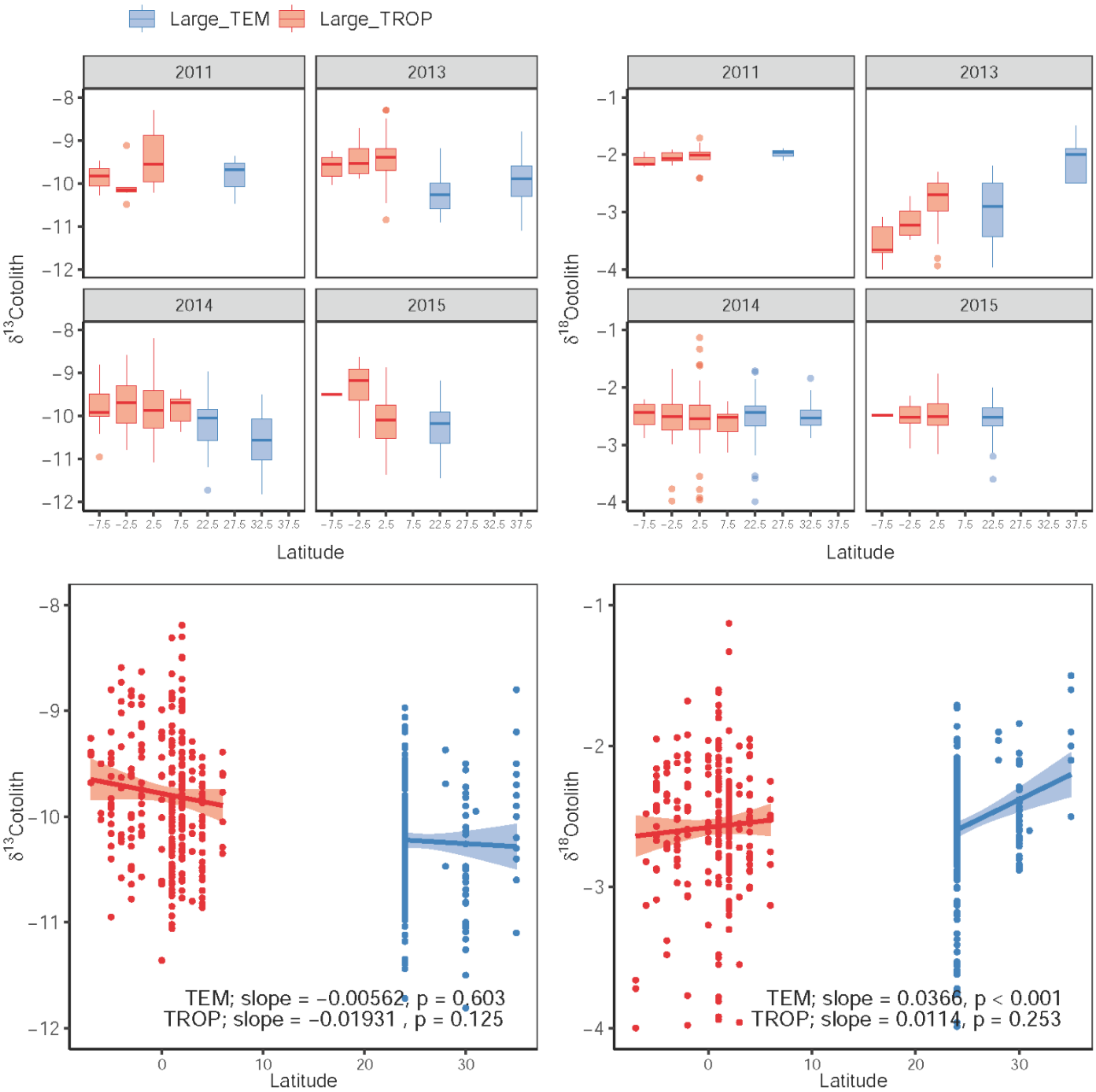

Fig. S7. Connectivity of juvenile yellowfin tuna. Exploratory analysis. Relationship between stable isotope ratios and latitude for large juveniles by temperate area (TEM) and tropical area (TROP). The blue and red shade is as in Fig. S1.

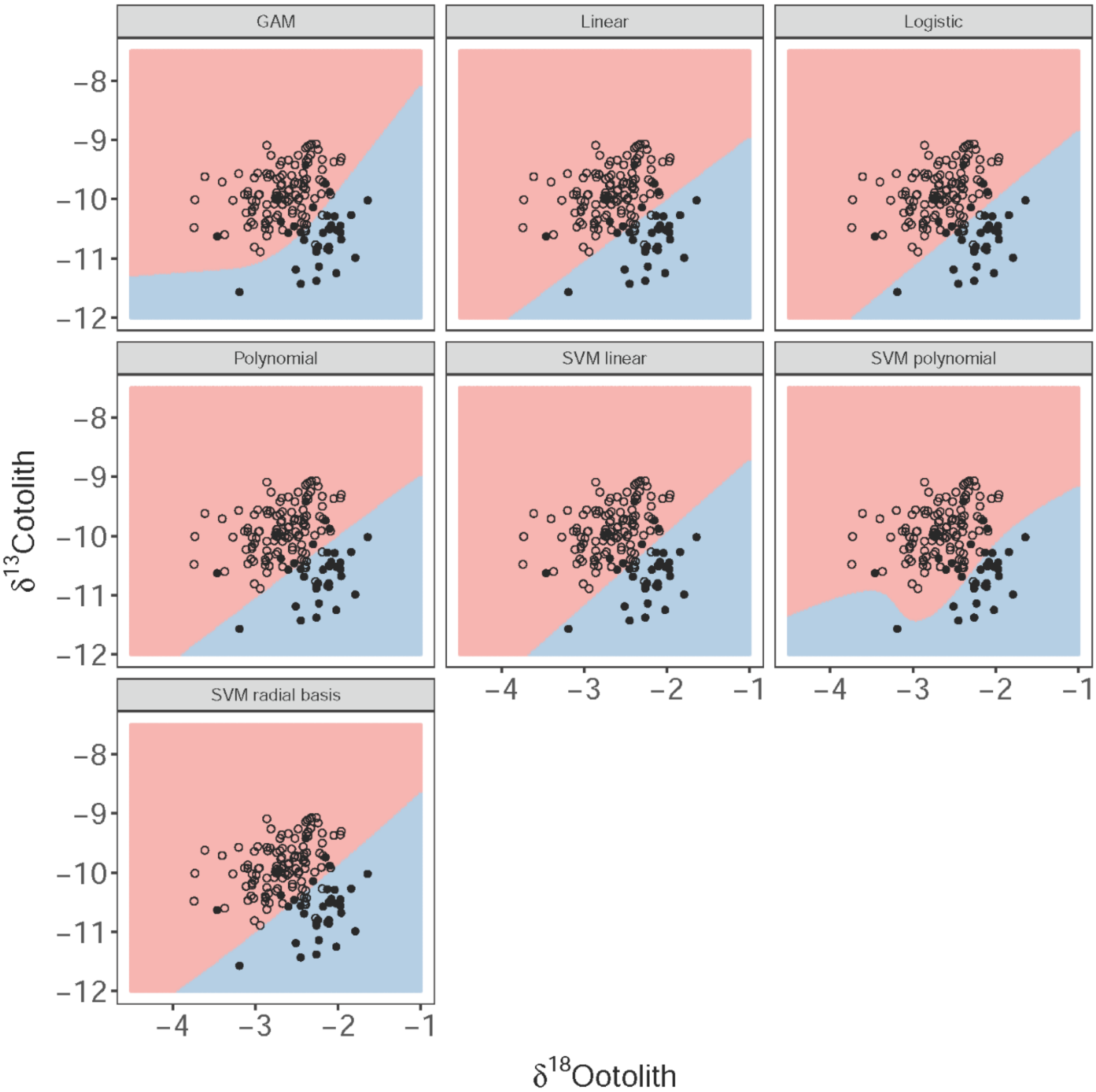

Fig. S8. Scatter plot of otolith oxygen ($\delta^{18}O_{otolith}$) and carbon ($\delta^{13}C_{otolith}$) of small juvenile yellowfin tuna (*Thunnus albacares*) by area in the western and central Pacific Ocean. Solid and open circles indicate the specimens caught in temperate and tropical areas, respectively. The red and blue-shaded areas represent the classification (estimated nursery ground) of temperate and tropical areas, respectively, based on multiple models, including support vector machine (SVM) with three types of kernel function (linear, polynomial, and radial basis); generalized additive model (GAM); and linear, polynomial, and logistic functions.

Table S1. Three oceanographic conditions (0: neutral, 1: El Niño, and 2: La Niña) in each month from 2005 to 2007 and 2011 to 2015

Year / Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2005	0	0	0	0	0	0	0	0	0	2	2	2
2006	2	2	2	0	0	0	0	0	0	0	0	0
2007	0	0	0	2	2	2	2	2	2	2	2	2
2011	2	2	2	0	0	0	0	0	0	0	0	0
2012	0	0	0	0	0	0	0	0	0	0	0	0
2013	0	0	0	0	0	0	0	0	0	0	0	0
2014	0	0	0	0	0	1	1	1	1	1	1	1
2015	1	1	1	1	1	1	1	1	1	1	1	1

Table S2. Classification success rates (%) of small juvenile yellowfin tuna (*Thunnus albacares*) in the western and central Pacific Ocean based on multiple models, including SVM with three types of kernel function (linear, polynomial, and radial basis); generalized additive model (GAM); and linear, polynomial, and logistic functions

model	Number of specimens in TROP		Classification success rate (%)	Number of specimens in TEM		Classification success rate (%)	Total classification rate (%)
	estimated	observed		estimated	observed		
Small juveniles							
SVM-linear	108	114	94.7	27	36	75.0	90.0
SVM-quadratic	113	114	99.1	24	36	66.7	91.3
SVM-radial basis	106	114	93.0	28	36	77.8	89.3
GAM	110	114	96.5	26	36	72.2	90.7
Linear	108	114	94.7	27	36	75.0	90.0
Quadratic	108	114	94.7	26	36	72.2	89.3
Logistic	108	114	94.7	27	36	75.0	90.0