# Spatial and seasonal variations of stable isotope ratios of particulate organic carbon and nitrogen in the surface water of the Kuroshio

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#### Abstract

Stable isotope ratios of carbon and nitrogen ( $\delta^{13}$ C and  $\delta^{15}$ N) in the particulate organic matter (POM) of the euphotic layer were seasonally investigated in the vicinity of the Kuroshio from 2008 to 2019 (n = 490). Generalized linear models (GLMs) showed significant seasonality of  $\delta^{13}$ C and  $\delta^{15}$ N of POM in the coastal (between Japan Main Island and the northern edge of the Kuroshio) and offshore (Kuroshio and more southern parts) areas. Seasonal climatological  $\delta^{13}$ C estimated based on the GLMs and seasonal median values of the environmental parameters was the highest in summer (-22.4 ± 0.2-22.9 +- 0.2in winter in both areas (-23.9 +- 0.2and offshore areas, respectively). Seasonal climatological  $\delta^{15}$ N showed different spatial variations from spring to summer. The  $\delta^{15}$ N value was the lowest during winter (0.8 ± 0.4seasons in the coastal area (~3nitrate originating in the deep-sea water was the main source of new production from spring to autumn. In contrast,  $\delta^{15}$ N in the offshore areas decreased from spring (2.6 ± 0.4suggesting that the significant contributions of atmospheric deposition and nitrogen fixation in the summer were similar to those around Hawaii. Therefore, the nitrogen sources for biological production were different between the areas and seasons in the vicinity of the Kuroshio.

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14	Running title: Stable isotope ratios of POM in the Kuroshio
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## 16 Key Points:

17	•	Stable isotope ratios of carbon and nitrogen in particulate organic matter (POM) are
18		seasonally investigated in the vicinity of the Kuroshio
19	•	Generalized linear models show significant seasonality of $\delta^{13}C$ and $\delta^{15}N$ in the POM in
20		coastal and offshore areas
21	•	Atmospheric deposition and nitrogen fixation are contributed to primary production in
22		the summer of the offshore area
23		

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43 Keywords: Particulate organic matter, Kuroshio,  $\delta^{13}$ C,  $\delta^{15}$ N, N<sub>2</sub> fixation, diatoms

## **1. Introduction**

46	The ocean absorbs large amounts of carbon and nitrogen, which have continuously
47	increased after the industrial revolution [Archer et al., 2009; Duce et al., 2008; Fowler et al.,
48	2013]. To detect the variations of the carbon and nitrogen dynamics in the ocean, the stable
49	isotope ratios of carbon and nitrogen are useful tracers [Gruber et al., 1999; Keeling, 1979;
50	Sigman et al., 2009; Sonnerup et al., 1999]. The proportion of <sup>13</sup> C to <sup>12</sup> C in soluble carbon
51	dioxide in seawater has linearly decreased at a rate of 0.2–0.1‰ decade <sup>-1</sup> with an increase in the
52	carbon dioxide concentration in the atmosphere [Gruber et al., 1999; Sonnerup et al., 1999].
53	The long-term linear changes in the ratio of <sup>15</sup> N to <sup>14</sup> N have been rarely detected in the previous
54	studies of the ocean [Chiba et al., 2012; Christensen and Richardson, 2008], but the ratio of <sup>15</sup> N
55	to <sup>14</sup> N in the coral skeleton-bound organic matter is decreasing with an increase in the
56	anthropogenic nitrogen deposition in this century [Ren et al., 2017].
57	The Kuroshio is the western boundary current of the North Pacific, and affects the long-
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58 59 60 61	term anthropogenic changes in the nitrogen and carbon dynamics [ <i>Ishii et al.</i> , 2011; <i>Watanabe et al.</i> , 2005]. The dynamic also varies seasonally with the physical processes [ <i>Kodama et al.</i> , 2014b]; the deep convection occurs in winter, while strong stratification occurs in summer. With strong stratification and increase in the surface water temperature, there is an abundance of
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ground for many types of small pelagic fishes, *e.g.* sardine (*Sardinops melanostictus*), and
anchovy (*Engraulis japonicus*), in spring [*Ohshimo et al.*, 2019], and migratory tunas, *e.g.*pacific bluefin tuna (*Thunnus orientalis*), yellowfin tuna (*T. albacares*), and skipjack tuna
(*Katsuwonus pelamis*), in the summer [*Tanaka et al.*, 2019; *Tawa et al.*, 2020].

72 The knowledge of the primary production processes in the vicinity of the Kuroshio is 73 limited. In general, determination of the stable isotope ratios of the phytoplankton can be 74 beneficial in understanding the primary production processes. For example, *Mino et al.* [2020] 75 investigated the seasonal variations in the stable isotope ratios of the settling particulate organic 76 matter (POM) collected outside the Kuroshio (station S1: 30°N, 145°E) with sediment traps and 77 reported that the nitrate supplied by deep-sea water accounted for  $\sim 90\%$  of the annual new 78 production in this area and the remnant corresponded to the biological nitrogen fixation. A 79 mixture of living and nonliving organic matter exists in the real ocean and it is difficult to 80 separate these [Volkman and Tanoue, 2002]; therefore POM, occasionally named seston, is 81 conventionally treated as the proxy of phytoplankton. Stable isotope ratios of the POM are also 82 useful in understanding the grazing food chain, and subsequently the marine ecosystem. The 83 nitrogen isotope ratio of the POM is typically reflected in that of the large predators [Aita et al., 84 2011; Horii et al., 2018]. Ohshimo et al. [2019] determined the carbon and nitrogen isotope 85 ratios in the muscle of the pelagic forage fish and squid in the western North Pacific and reported 86 that their nitrogen isoscape possibly reflected the balance of nitrogen fixation and denitrification, 87 but did not investigate the stable isotope ratios of the POM. In the vicinity of the Kuroshio, fifty-88 year repeat observations were conducted along 137°E [Oka et al., 2018], but the stable isotope 89 ratios of the marine organisms including those of the POM were not measured in these 90 observations.

91 Ohshimo et al. [2019] determined both the high and low nitrogen isotope ratios in pelagic 92 forage fish and squid found in the Kuroshio area, and reported that detailed analysis of the stable 93 isotope ratios of the POM were necessary for understanding the grazing food chain and marine 94 ecosystem of this area. Therefore, in this study, carbon and nitrogen stable isotope ratios of the 95 POM in the area adjacent to the Kuroshio water are determined in the over decadal seasonal 96 investigations. The seasonal and yearly variations in the stable isotope ratios of the POM are 97 examined using environmental factors and empirical models, followed by the evaluation of the 98 seasonal climatological values of the stable isotope ratios. The goal of this study is to provide 99 insights into the carbon and nitrogen dynamics in the Kuroshio area, in particular, the nitrogen 100 source for the primary production in this area.

101

#### 102 **2.** Materials and methods

#### 103 **2.1. Observation**

104 POM and environmental data were collected from 2008 to 2019 along the 138°E 105 coordinate between 34°30'N and 27°N (named as "O-line") [Figure 1] using R/V Soyo-maru of 106 Japan Fisheries Research and Education Agency. The Kuroshio usually flows 32-33°N at 107 138°E; however, after the Kuroshio large meander in 2017, it flows between 30–32°N [Figure 108 1]. As the Kuroshio shows spatial contrast in the biogeochemical conditions between its 109 northern and southern parts [Kodama et al., 2014b], the observation areas were divided as the 110 coastal (>13 nautical miles north to the Kuroshio axis) and offshore (the Kuroshio and southern 111 part of the Kuroshio axis) areas. The position of the Kuroshio axis was defined as the monthly 112 mean values of the position in the Quick Bulletin of Ocean Conditions published by the Japan 113 Coast Guard (www1.kaiho.mlit.go.jp/KANKYO/KAIYO/qboc/index E.html).

114	The POM sampling was conducted seasonally from 2008 to 2015, and the observations
115	were limited to the summer (August or September) and winter (January or February) after 2016
116	[Figure 1]. In summary, the observations were conducted at 23 stations in winter, 30 stations in
117	spring (March, April, or May), 28 stations in summer, and 21 stations in autumn (October or
118	November). After measuring the vertical distributions of the photosensitive available radiation
119	(PAR) using a discreate PAR sensor (PRR-800 or INF-300, Biospherical Instruments Inc., San
120	Diego, California, USA), the water samples were collected using the 12-L X-Niskin bottles
121	attached directly to the cable at five different water depths for which the PAR values were 100,
122	50, 25, 10, and 1% relative to the surface until May 2016. The sampling depth was fixed after
123	May 2016 within the upper 50 m. The vertical profiles of temperature and salinity were
124	measured using a conductivity-temperature-depth (CTD) sensor (SBE9plus, Sea-Bird Scientific,
125	Washington, USA). The discrete samples for determining nutrient and chlorophyll $a$
126	concentrations were obtained using the Niskin bottles attached to the CTD-CMS (Carousel
127	Multisampling System). Vertical resolution of the discrete samples (nutrients and chlorophyll <i>a</i>
128	concentrations) was 10 m depth. As the environmental parameters determined using the POM
129	samples were limited, the nearest depth data collected by the CTD-CMS was adopted for the
130	following statistical analysis. The details of the nutrient and chlorophyll <i>a</i> analyses were
131	previously reported in the literature [Kodama et al., 2014b]. To treat the common-logarithm
132	transformed values, the nitrate concentration below 0.01 $\mu$ M was set as 0.01 $\mu$ M.
133	Diatom abundance was investigated at a depth of 10 m for some stations. One liter of
134	seawater samples were collected and fixed with glutaraldehyde solution. The carbon amount of
135	the diatom cells was calculated based on the lengths of the major axes and shapes [Menden-
136	Deuer and Lessard, 2000]. The number of one of the major dinitrogen fixation cyanobacteria,

*Trichodesmium,* was also counted. These were sporadically present regardless of the season and
mostly negligible; therefore, these results are not included.

139

#### 140 **2.2.** Isotope analysis

To measure the carbon and nitrogen stable isotope ratios of the POM, three liter of seawater samples were filtered using a pre-combusted (450 °C, 6 h) glass fiber filter (Whatman GF/F, Whatman). The amount of seawater was < 3 L in some case when the filtration time was long. After filtration on board, the samples were kept frozen (-30 °C). In the on-land laboratory, the samples were dried in a dry oven (60 °C, overnight) and kept in a desiccator until

146 decarboxylation.

147 Before measuring the isotope ratio, the samples were exposed to HCl fumes for more 148 than two hours to remove the carbonate salt. After drying again in the dry oven, the colored 149 surfaces of the glass filters were scraped off, and their stable isotope ratios were measured using 150 an isotopic ratio mass spectrometer (MAT 252, Thermo Fisher Scientific (Waltham, 151 Massachusetts, USA) till October 2013, and thereafter, using Isoprime 100 (Elementar, 152 Langenselbold, Germany). The stable isotope ratios of carbon and nitrogen were calibrated 153 using the curves obtained with L-alanine during the measurements. The isotopic compositions of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) are expressed by following equation: 154

155 
$$\delta \mathbf{X} = (\mathbf{R}_{\text{sample}} / \mathbf{R}_{\text{standard}} - 1.0) \times 1000, \tag{1}$$

- 156 where X,  $R_{sample}$ , and  $R_{standard}$  are the target elements (C or N), and the heavy (<sup>13</sup>C and <sup>15</sup>N) to
- 157 light (<sup>12</sup>C and <sup>14</sup>N) isotope ratios of the sample and standard, respectively. The standards
- 158 included atmospheric N<sub>2</sub> for nitrogen and Vienna Pee Dee Belemnite for carbon. The precision

159	of the analysis varied with the amounts of carbon and nitrogen and between the spectrometers,
160	but the values were within 0.2‰ for $\delta^{13}$ C and 1‰ for $\delta^{15}$ N. Considering the quality of the
161	standards, the $\delta^{13}$ C and $\delta^{15}$ N values of each sample were rounded off to one decimal place.
162	The $\delta^{13}$ C and $\delta^{15}$ N values of the POM were sometimes the outlier values. Therefore, the
163	mean and standard deviations (sd) were calculated for $\delta^{13}C$ and $\delta^{15}N$ values for all the samples,
164	and the samples whose $\delta^{13}$ C or $\delta^{15}$ N values differed by >3 × sd from the mean values were
165	eliminated. This quality control process was performed twice. In addition, the samples for
166	which the $\delta^{13}$ C and $\delta^{15}$ N values were available, but the environmental parameters were not
167	recorded were removed. As a result, 490 samples out of 526 samples were used in the following
168	statistical analyses. The breakdown of seasonality was as follows: the numbers in winter, spring,
169	summer, and autumn were 108, 147, 133, and 102, respectively. Our data was in the
170	supplemental information.

#### 172 2.3. Statistical Analysis

173 Statistical analysis was conducted using R version 4.0 [*R Core Team*, 2020]. Generalized 174 linear models (GLMs) were applied to assess the variables that predicted the  $\delta^{13}$ C and  $\delta^{15}$ N 175 values for seasonal and yearly variations. GLM is one of the empirical models for understanding 176 the relationships between explanatory variables and response variables. The models are 177 described as follows:

$$178 \quad \delta X \sim N(\mu, \sigma^2) \tag{2}$$

 $179 \quad \delta X \sim glm[f(Y) + f(A):f(Sea) + f(A) + f(Sea) + T + Sal + log-Chl + log-Nit + Dep + K]$ (3)

 $180 \qquad \delta X_{10m} \sim glm[f(Y) + f(A):f(Sea) + f(A) + f(Sea) + T + Sal + log-Chl + log-Nit + log-Diat + K]$ (4)

where N,  $\mu$  and  $\sigma^2$  are the normal distribution, mean, and variance, respectively.  $\delta X$  correspond 181 to  $\delta^{13}$ C or  $\delta^{15}$ N, and  $\delta X_{10m}$  refers to the values at a depth of 10 m, at which the phytoplankton 182 183 community structures are observed. Y, A, Sea, T, Sal, log-Chl, log-Nit, Dep, log-Diat, and K 184 indicate the sampling year, area (coastal or offshore), season (winter, spring, summer, or 185 autumn), temperature, salinity, common-logarithm transformed chlorophyll a concentration 186 (abbreviated as log-chlorophyll, except in the equations), common-logarithm transformed nitrate 187 concentration (log-nitrate, except in the equations), sampling depth, common-logarithm 188 transformed carbon amounts of diatoms (log-diatom, except in the equations), and latitude of the 189 Kuroshio axis, respectively. The letter "f" indicates a categorical variable. For the interaction term between the area and seasons, f(A): f(Sea), it was assumed that the intercept of  $\delta^{13}C$  or  $\delta^{15}N$ 190 191 is different between the areas. In these analyses, it was assumed that the environmental parameters were linearly correlated to  $\delta^{13}$ C or  $\delta^{15}$ N; hereafter, the model is described as the 192 193 linear function GLM. The models using the full data (eq. 3) were considered to be more reliable 194 rather than those limited by the 10 m depth (eq. 4), because the latter had only a small number of 195 data sets, which generated bias in the annual and seasonal sampling. Considering the nonlinear relationship between the explanatory variables and  $\delta^{13}$ C or  $\delta^{15}$ N, the following GLMs were also 196 applied to  $\delta^{13}$ C or  $\delta^{15}$ N: 197

198 
$$\delta X \sim \text{glm}[f(Y) + f(A):f(\text{Sea}) + f(A) + f(\text{Sea}) + p(T, 4) + p(\text{Sal}, 4) + p(\text{log-Chl}), 4) + p(\text{log-Nit}, 4)$$

(5)

199 + 
$$p(Dep, 4) + p(K, 4)$$
]

Here, p(Z, 4) denotes the numerical explanatory variables (Z) applied as quartic functions

201 (degree of 4). Quartic function was selected to avoid overfitting. Hereafter, the model described

202 by eq. 5 is named as the quartic function GLM.

203	The generalized variance inflation factor (GVIF) was calculated using the "car" package
204	[Fox and Weisberg, 2018], and the highest GVIF parameters adjusted using the degree of
205	freedom were <10. The explanatory variables and final model descriptions were selected on the
206	basis of the Akaike Information Criterion (AIC). The "emmeans" package [Lenth, 2020] was
207	used to calculate the least squares mean (lsmean) values and standard errors of the yearly
208	variations based on the AIC-selected GLMs. The effects of the parameters were visualized using
209	"response.plot2" function in the "biomod2" package [Thuiller et al., 2020].

211 **3. Results** 

#### 212 **3.1.** Seasonality of environmental parameters

213 The water temperature was the highest in summer but some cold water (<18 °C) was 214 observed in the coastal area at the subsurface [Figure 2]. In summer and autumn, less-saline 215 water (<34) was also found in the area [*Figure 2*], which was attributed to the warm coastal 216 water input (>22 °C). Contrary to the coastal areas, warm and high-salinity water was found in 217 the offshore areas. In the offshore area, water was less-saline in summer than that in winter and 218 spring [Figure 2]. The chlorophyll a concentration in the coastal area increased in the spring, 219 and decreased in summer [Figure 2]. In the offshore area, chlorophyll a concentrations in the 220 winter and spring were identical. Nitrate concentration was negligible near the surface from 221 spring to autumn in the offshore area and during summer in the coastal area [Figure 2]. At the 222 base of the eutrophic layer (1% light depth), the nitrate was not depleted even in summer. The 223 diatom abundance at a depth of 10 m was the highest during the spring in the coastal area, and it 224 was higher than that of the offshore area during all seasons [Figure 2]. In the offshore area, the 225 diatom abundance was identical in winter and spring, but decreased in summer and autumn.

#### 227 **3.2.** Isotope ratios

The  $\delta^{13}$ C and  $\delta^{15}$ N values of POM varied between -28.7 and -20.3‰ for  $\delta^{13}$ C and 228 between -4.0 and 8.0% for  $\delta^{15}N$  (*n* = 490) [*Figure 3*]. When the data was divided into the area 229 and season, the seasonal variations of both  $\delta^{13}$ C and  $\delta^{15}$ N values were significant for both the 230 coastal and offshore areas (ANOVA, p < 0.01). The 95% probability ellipses showed that both 231 the  $\delta^{13}$ C and  $\delta^{15}$ N values varied most widely during spring in the coastal area, and those in 232 233 summer and autumn were within the variations observed during spring [Figure 3]. During winter in the coastal area,  $\delta^{13}$ C was within the range observed in the spring, but  $\delta^{15}$ N was slightly 234 lower. In the offshore area, both  $\delta^{13}$ C and  $\delta^{15}$ N values varied most widely in the summer, and 235 those during autumn were within the ranges observed in summer. The  $\delta^{13}$ C and  $\delta^{15}$ N values in 236 237 winter and spring were slightly lower and higher, respectively, in comparison to those during 238 summer.

239

#### 240 **3.3.** Relationship between environmental parameters and isotope ratios

Significant (*F*-test, p < 0.05) linear relationships between  $\delta^{13}$ C and several environmental factors, *i.e.* temperature, salinity, and log-nitrate were observed [*Figure 4*]. The coefficients and intercept of the linear regression between  $\delta^{13}$ C and temperature were  $0.060 \pm 0.013$  (‰°C<sup>-1</sup>) and -25.3 ± 0.3‰, respectively, and those between  $\delta^{13}$ C and salinity were -2.1 ± 0.20 (‰) and 48.7 ± 6.6‰, respectively. The coefficient of determination ( $r^2$ ) shows salinity was the strongest explanatory parameter ( $r^2 = 0.197$ ), and temperature and log-nitrate were weak parameters ( $r^2 =$ 0.037 and 0.026, respectively). There was no significant relationship between  $\delta^{13}$ C and logchlorophyll (p > 0.2), and the latitude of the Kuroshio axis [*Figure 4*]. There was a positive relationship between  $\delta^{13}$ C and log-diatom [*Figure 4*].

Significant (*F*-test, p < 0.05) linear relationships were observed between  $\delta^{15}$ N and both salinity and log-nitrate [*Figure 5*], but both relationships were weak based on the coefficients of determination ( $r^2 = 0.008$  and 0.02 for salinity and log-nitrate, respectively). Significant linear relationships were not observed between  $\delta^{15}$ N and other environmental factors such as temperature, log-chlorophyll, latitude of Kuroshio axis, and log-diatom. The  $\delta^{15}$ N value of the POM increased with an increase in the temperature below 20 °C, but in warm water, the linear trend was not significant [*Figure 5*].

257

#### 258 **3.4.** Results of GLMs

259 The descriptions of the least-AIC GLMs are included in *Table 1*. The sampling depth was selected neither for  $\delta^{13}$ C nor  $\delta^{15}$ N in all the models. The selected parameters were different 260 in each model, and for  $\delta^{15}$ N of the full data model with linear function, only temperature was 261 262 selected as the environmental parameter. Seasonal and spatial differences including their 263 interaction term were selected in the linear function models, while their interaction term was not included in the quartic function models. The coefficient of determination of the GLMs using the 264 265 quartic function was higher than those using the linear functions. Yearly variation was not selected only in the model of  $\delta^{13}C$  at 10-m depth. 266

267 The responses of the numerical explanatory variables in the least-AIC GLMs are shown 268 in *Figure 6*. In the full data set model using only the linear functions for  $\delta^{13}$ C, the negative 269 effects to  $\delta^{13}$ C were observed for temperature, salinity, log-nitrate, and latitude of the Kuroshio

axis, while the positive effect was observed for log-chlorophyll [*Figure 6*]. These relationships were statistically significant, except for log-nitrate (p < 0.05). In the full data set model considering the nonlinear effects for  $\delta^{13}$ C, the effect of the latitude of the Kuroshio axis was different from that with the linear function:  $\delta^{13}$ C showed higher values when the large mender occurred [*Figure 6*]. The responses of the other parameters were not considerably different from those of the linear functions [*Figure 6*]. The GLM for  $\delta^{13}$ C at 10 m depth showed that logdiatom significantly and positively affected the  $\delta^{13}$ C values [*Figure 6*].

For  $\delta^{15}$ N, only temperature was selected and found to positively affect  $\delta^{15}$ N in the GLM 277 278 employing linear functions, while temperature, salinity, log-chlorophyll, and log-nitrate were 279 selected in the GLM including the quartic functions [Figure 7]. The temperature showed a 280 unimodal distribution, and the peak of the response was observed at ~20 °C [Figure 7]. The unimodal distribution was also observed for log-nitrate; the  $\delta^{15}$ N value decreased when the 281 282 nitrate concentration was greater than the sub-micromolar levels [Figure 7]. The increases in log-chlorophyll and salinity showed negative effects on  $\delta^{15}$ N [*Figure 7*]. Log-diatom was 283 selected and showed negative effects on  $\delta^{15}$ N [*Figure 7*], but the coefficient was not significantly 284 285 different from 0.

After considering the environmental parameters, spatial, yearly, and seasonal differences were still significant, except the spatial difference of  $\delta^{13}$ C estimated from the quartic function-GLM [*Table 1*]. After calculating the yearly Ismean  $\delta^{13}$ C and  $\delta^{15}$ N values, the reduced sampling frequency after 2017 resulted in large standard errors in  $\delta^{13}$ C and  $\delta^{15}$ N, which made the yearly Ismean values unreliable [*Figure 8*]. In addition, the effects of the Kuroshio large mender after 2018 [*Figure 1*] also made the Ismean  $\delta^{13}$ C unreliable, in particular, for the values based on the quartic function GLM [*Figure 8*]. Except these unreliable yearly values (*i.e.* after 2017), Ismean

(± SE)  $\delta^{13}$ C values ranged between -25.2 ± 0.1‰ (2013) and -23.4 ± 0.2‰ (2008) for the linear function GLM, and between -24.9 ± 0.2‰ (2013) and -23.6 ± 0.2‰ (2008) for the quartic function GLM. In contrast to  $\delta^{13}$ C,  $\delta^{15}$ N varied between 0.6 ± 0.2‰ (2013) and 3.1 ± 0.2‰ (2014) for the linear function GLM, and between 2.0 ± 0.3‰ (2013) 4.2 ± 0.3‰ (2014) for the quartic function GLM. In 2013, both the Ismean values of  $\delta^{13}$ C and  $\delta^{15}$ N were the lowest. The  $\delta^{13}$ C and  $\delta^{15}$ N values had no significant linear relationships to the year when the data after 2017 were removed.

300 For the calculation of the seasonal and spatial lsmean values, the environmental 301 parameters except season and area were fixed. However, in the observations, the environmental 302 parameters showed some degrees of collinearity to the season and/or areas. Therefore, the seasonal and spatial "climatological"  $\delta^{13}$ C and  $\delta^{15}$ N values were calculated using the least-AIC 303 304 GLMs and median values of the environmental parameters of each season and area. The year was fixed as 2012, when both the Ismean  $\delta^{13}$ C and  $\delta^{15}$ N values were neither maximum nor 305 minimum. As a result, significant spatial difference in the seasonality of  $\delta^{13}$ C and  $\delta^{15}$ N was 306 307 detected regardless of the linear and quartic function GLMs [Figure 9]. Because the coefficients of determination ( $r^2$  values) were greater for the quartic function GLM than those for the linear 308 309 function GLM [Table 1], the numerical values hereafter correspond to the results of the quartic function GLM. In the coastal area, both the climatological  $\delta^{13}$ C and  $\delta^{15}$ N values were the lowest 310 in winter (-23.9  $\pm$  0.2% [mean  $\pm$  SE] and 0.8  $\pm$  0.4%, respectively). The  $\delta^{15}$ N values were 311 higher than 2.5% and similar in the other three seasons  $(2.7 \pm 0.4, 2.7 \pm 0.4 \text{ and } 3.2 \pm 0.4\% \text{ in})$ 312 spring, summer, and autumn, respectively). The  $\delta^{13}$ C values were highest in summer (-22.4 ± 313 314 0.2‰), and those during spring and autumn were similar (-23.1  $\pm$  0.2 and -23.2  $\pm$  0.2, respectively). In the offshore area, the seasonal  $\delta^{13}$ C and  $\delta^{15}$ N values varied in a narrower range 315

than those in the coastal area. The  $\delta^{13}$ C values were lowest in winter (-24.3 ± 0.2‰) and highest in summer (-22.9 ± 0.2‰), corresponding to the same trend observed in the coastal area. However, the  $\delta^{15}$ N value was the highest in spring (2.6 ± 0.4‰) and lowest in summer (0.9 ± 0.4‰). Moreover, the  $\delta^{15}$ N value in the offshore area during winter (1.6 ± 0.4‰) was higher than that in the coastal area.

321

#### 322 **4. Discussion**

### 323 4.1. Comparison of the previous studies

In the upstream of the Kuroshio and the East China Sea, the horizontal gradients of  $\delta^{13}$ C 324 and  $\delta^{15}$ N of the POM were reported [*Wu et al.*, 2003] to range from -19 to -31‰ for  $\delta^{13}$ C and 325 from 0.7 to 9.5% for  $\delta^{15}$ N in autumn. Saino and Hattori [1987] reported only the  $\delta^{15}$ N value in 326 327 the Philippine Sea (western North Pacific Subtropical Gyre), i.e., -1.1‰ at the surface, and *Minagawa et al.* [2001] reported values between -21.6 and -24.4‰ for  $\delta^{13}$ C and between ~1‰ 328 and ~4‰ for  $\delta^{15}$ N in the East China Sea during summer. *Takai et al.* [2007] reported the  $\delta^{13}$ C 329 and  $\delta^{15}$ N values of the POM ranging from -24.2 to -19.1 and 0.7 to 6.8, respectively, around the 330 331 Izu Peninsula, corresponding to the coastal area of our study but including the ashore area. Mino et al. [2020] also reported the seasonal variations in the northern edge of the western North 332 Pacific Subtropical Gyre. In their observations, the  $\delta^{15}$ N values of the POM ranged between 1.5 333 and 2.6‰ at 0–100 m depth [*Mino et al.*, 2020], but *Mino et al.* [2020] did not report the  $\delta^{13}$ C 334 335 values. The ranges for these values are consistent with the results presented herein. Therefore, 336 considering that results in the present study are based on the over-decadal seasonal repeated observations, the climatological  $\delta^{15}$ N and  $\delta^{13}$ C values herein [*Figure 9*] can be treated as the 337

seasonal climatological values in the Kuroshio area, the western boundary current of the NorthPacific.

340 The yearly variations in this study did not show any significant long-term trends for both  $\delta^{13}$ C and  $\delta^{15}$ N [*Figure 8*]. This was different from the previous studies that inferred the values 341 from the  $\delta^{13}$ C content of the fish muscles [Lorrain et al., 2020], which suggested that the  $\delta^{13}$ C 342 content of the POM decreased linearly in the 21<sup>st</sup> century. The study period in the present 343 344 investigation is shorter than the previous studies [Lorrain et al., 2020], and decadal variations of 345 biogeochemical processes are pointed in the Kuroshio [Oka et al., 2019; Watanabe et al., 2005]. Therefore, further long-term monitoring of  $\delta^{13}$ C and  $\delta^{15}$ N is necessary for understanding their 346 347 variation trends in the POM, and for determining the anthropogenic effects of carbon and 348 nitrogen dynamics in the Kuroshio.

349

#### 350 4.2. Carbon dynamics in the vicinity of the Kuroshio

Both  $\delta^{13}$ C and  $\delta^{15}$ N values of the POM are different from those of the phytoplankton 351 because POM contains heterotrophic small planktons and detritus, but it is considered that  $\delta^{13}C$ 352 and  $\delta^{15}$ N values of POM are largely dependent on those of the phytoplankton in open water. The 353  $\delta^{13}$ C value of the phytoplankton depends on the CO<sub>2</sub> concentration in water, growth rate, and cell 354 355 geometry of each species [Popp et al., 1998]. The growth rate of the phytoplankton depends on 356 temperature, nutrient concentration, and light conditions [Cross et al., 2015], and CO<sub>2</sub> 357 concentration in water depends on the temperature, atmospheric CO<sub>2</sub> concentration, and phytoplankton growth. As the result, the  $\delta^{13}$ C value of the POM or phytoplankton becomes 358

higher with an increase in the temperature [*Goericke and Fry*, 1994; *Wada et al.*, 2012], and with
the rapid growth of the phytoplankton [*Laws et al.*, 1995].

However, the  $\delta^{13}$ C response to temperature was weak or negligible in the present study 361 362 contrary to that observed in the previous studies [Figures 4 and 6]. The coefficient of simple linear regression analysis between temperature and  $\delta^{13}$ C [*Figure 4*] was  $0.06 \pm 0.01$  (‰ °C<sup>-1</sup>). 363 364 which was smaller than that obtained by the global data estimation  $(0.17 \pm 0.04)$  [Goericke and 365 Fry, 1994] and in western North Pacific (0.25) [Wada et al., 2012]. The seasonal variations indicated that the  $\delta^{13}$ C value of the POM was high in warm water (for example,  $\delta^{13}$ C in the 366 367 offshore area during summer), but the other factors such as salinity and chlorophyll a concentration more significantly affected the  $\delta^{13}$ C variations in the Kuroshio. The other possible 368 369 mechanism for the weak or negligible temperature response was the gravitational downward flux 370 of the POM from the warm surface water to cold subsurface water. When the downward flux was active and heterogenous both in time and space, the relationship between  $\delta^{13}$ C and *in situ* 371 temperature was weaker than the empirical values, because the high  $\delta^{13}$ C of the POM produced 372 in the warm surface layer was mixed with the low  $\delta^{13}$ C of POM produced in the cold subsurface 373 374 layer.

The highest  $\delta^{13}$ C value was observed during spring in the coastal area, with a high diatom carbon abundance. The carbon isotopic fractionation of the diatoms is larger than that of the cyanobacteria [*Popp et al.*, 1998]. Therefore, it was considered that rapid growth of diatoms in the blooms afforded the highest  $\delta^{13}$ C in the POM during spring in the coastal area. In the coastal area of the Kuroshio, nutrient supply during winter was significantly higher than that in the offshore area [*Kodama et al.*, 2014b]. Thus, the more diatom blooms were observed compared to those in the offshore area, and the  $\delta^{13}$ C value of the POM in the coastal area was higher than

that in the offshore. The positive coefficient of log-chlorophyll for  $\delta^{13}$ C in the GLM also

383 supported that  $\delta^{13}$ C of POM elevates during the rapid phytoplankton growth. These observations

384 suggested that carbon sequestration during spring in the coastal area was active, and  $\delta^{13}$ C of

385 POM varied with the phytoplankton community structure in the real ocean.

386 The salinity decreased during summer and autumn in the coastal area and showed a negative effect on  $\delta^{13}$ C [*Figures 4 and 6*]. Thus, a high  $\delta^{13}$ C was observed in less-saline water 387 supply. In the estuary, the  $\delta^{13}$ C value of the POM is sometimes affected by the riverine POM 388 [*Bănaru et al.*, 2014]. The intercept of the simple linear relationship between salinity and  $\delta^{13}$ C 389 indicated that the  $\delta^{13}$ C value of the POM in original freshwater was  $48.7 \pm 6.6\%$  if the 390 relationship between salinity and  $\delta^{13}$ C was established only based on the results of mixing 391 riverine and oceanic POM. This estimated value was unrealistic: the  $\delta^{13}$ C of the riverine POM 392 393 was between -29.1‰ and -28.7‰ [Nagao et al., 2013; Nagao et al., 2010], between -12‰ and -28‰ at the Ise bay, which was near the observation area of the present study [Sugimoto et al., 394 2004], and -26.9  $\pm$  1.1‰ in the Tokyo bay [*Ogawa and Ogura*, 1997]. Therefore, the high  $\delta^{13}$ C 395 396 of the POM in the summer was not the result of the horizontal advective transport of the riverine 397 POM.

In addition to the POM, inorganic nutrients are also supplied by the riverine freshwater. With these nutrients, the high  $\delta^{13}$ C values of the POM in the estuary reported by the previous studies [*Ogawa and Ogura*, 1997; *Sugimoto et al.*, 2004] are due to the phytoplankton blooms (rapid growth) in the low-salinity nutrient-rich water. With low-salinity water, such a high- $\delta^{13}$ C POM is possibly advected from the estuary or eutrophic ashore areas to the investigated area in the present study. In fact, a high  $\delta^{13}$ C was observed in the ashore area near this investigated area by *Takai et al.* [2007]. Therefore, considering the  $\delta^{13}$ C values, the contribution of the riverine inorganic nutrients may be significant to the productivity in the coastal area of the Kuroshio.

The Kuroshio path also affected to  $\delta^{13}$ C value of the POM in the GLMs [*Figure 6*]. The 406 407 coefficients of the linear function GLM suggested that when the Kuroshio flowed near the coast, the  $\delta^{13}$ C value of the POM decreased, and when the Kuroshio large meander occurred, the  $\delta^{13}$ C 408 value of the POM increased. The quartic function GLM indicated that the  $\delta^{13}$ C value of the 409 410 POM was significantly higher when the Kuroshio large meander occurred, but considering the 411 yearly Ismean, the response to the Kuroshio path in the quartic function GLM was unreliable and 412 overfitted. Additional studies are necessary to understand the relationship between the Kuroshio path and  $\delta^{13}$ C. 413

414

#### 415 **4.3.** Nitrogen dynamics in the vicinity of the Kuroshio

The  $\delta^{15}$ N value of the POM varies with  $\delta^{15}$ N of the original nitrogen sources (nitrate. 416 ammonium, and N<sub>2</sub> fixation), and degree of isotopic fractionation. The rate of isotopic 417 418 fractionation of the phytoplankton is controlled by its growth rate [Wada and Hattori, 1990]. The highest  $\delta^{15}$ N value of the POM is produced under nitrate-depleted condition, in which the 419 nitrogen isotopic fractionation of nitrate does not occur [*Wada et al.*, 2012]. The lowest  $\delta^{15}$ N 420 421 value of the phytoplankton originates from nitrogen fixation [Minagawa and Wada, 1986]. The  $\delta^{15}$ N value of the nitrate is 3–5‰ in the vicinity of the Kuroshio [Sugimoto et al., 2009; 422 423 Yoshikawa et al., 2018]. In winter, vertical mixing occurs, and nitrate is present at a micromolar 424 level both in the coastal and offshore areas in the euphotic layer [Figure 2]. In spring, 425 stratification starts occurring, and the nitrate in the offshore depletes but is detectable at the sub-

micromolar level [*Figure 2*]. The  $\delta^{15}$ N value of the POM (phytoplankton) is lower than that of 426 427 the nitrate under conditions with sufficient nitrate [Fogel and Cifuentes, 1993; Montoya and *McCarthv*, 1995; *Wada*, 1980]. Therefore, the lower climatological  $\delta^{15}$ N values of the POM in 428 429 winter  $(0.8 \pm 0.4 \text{ and } 1.6 \pm 0.4\%)$  in the coastal and offshore areas, respectively, Figure 9) 430 compared to those during spring were owing to the difference in the nitrate, *i.e.*, nitrate 431 consumption was lower than nitrate supply in the winter, while nitrate consumption was greater 432 than nitrate supply in the spring. Similar phenomenon was reported by *Mino et al.* [2020]. The 433  $\delta^{15}$ N value of the POM in the sediment traps [*Mino et al.*, 2020] was higher than the values in water, but a decrease in the  $\delta^{15}$ N value of the POM in the winter was similar because of the 434 435 active nitrate supply due to deep mixing.

During summer, nitrate was largely depleted both in the coastal and offshore areas at the surface, but still remained at the base of the euphotic layer in the coastal area [*Figure 2*]. In addition, ammonium was depleted in the offshore area, while it remained in the coastal area at the nanomolar level [*Kodama et al.*, 2014a], and nitracline was shallower in the coastal area than in the offshore area [*Kodama et al.*, 2014b]. These observations suggested that the nitrateoriginating new production was active in the coastal waters, but not in the offshore water.

Two possible sources that decrease  $\delta^{15}$ N of the POM in the offshore area during summer are 1) nitrogen fixation and 2) wet and dry deposition of the nitrogenous nutrients from the atmosphere. First, the Kuroshio and western North Pacific are two of the "hotspots" of nitrogen fixation during summer [*Shiozaki et al.*, 2010; *Shiozaki et al.*, 2018; *Shiozaki et al.*, 2015]. In the microscopic observations at 10 m depth, *Trichodesmium* is not abundant but an important dinitrogen-fixation phytoplankton in the Kuroshio [*Shiozaki et al.*, 2018; *Shiozaki et al.*, 2015], which floats upward and accumulates at the surface [*Walsby*, 1992]. The  $\delta^{15}$ N of *Trichodesmium* 

449 ranges between -2.1 and 0.8‰ [*Minagawa and Wada*, 1986]. Therefore, the decrease in  $\delta^{15}$ N of 450 the POM during the winter was attributed to active nitrogen fixation.

451 Second, the wet and dry deposition of nitrogenous nutrient inputs from nitrogen fixation 452 and nitrate supply from deep water in the upstream of the Kuroshio are similar during summer [Kodama et al., 2011], and  $\delta^{15}$ N of the nitrate in rainwater is <0% in Japan [Wu et al., 2008]. 453 Therefore, the wet and dry deposition of nitrogenous nutrient inputs also decrease the  $\delta^{15}N$  of the 454 POM. Both factors decreased the  $\delta^{15}$ N of the POM in the offshore area during summer. In the 455 456 autumn, the mixing layer deepened, and the nitrate concentration again increased in the euphotic layer [*Figure 2*], and thus,  $\delta^{15}$ N value of the POM was a mixture of the summer values and 457 458 nitrate-originating values.

In the coastal area, the climatological  $\delta^{15}$ N value in the summer was similar to that during 459 460 spring, suggesting that the contributions of nitrogen fixation and atmospheric deposition were 461 small during the summer. In contrast, salinity decreased in the summer; therefore, riverine 462 inputs as well as nitrogen fixation and atmospheric depositions must be considered because 463 riverine inputs of the nitrogenous nutrients possibly comprise  $\sim 10\%$  of the nitrogenous nutrient 464 input in the coastal area [Kodama et al., 2014b]. The aforementioned relationship between salinity and  $\delta^{13}$ C also indicated that riverine inputs of the nitrogenous nutrients influenced the 465 productivity in the coastal area. However, the  $\delta^{15}$ N value of riverine nitrate varied from 0 to 8‰, 466 and decreased with an increase in the river discharge and was unstable [Sugimoto et al., 2011]. 467 The  $\delta^{15}$ N of ammonium was high (typically >10%), but the concentration of ammonium was 468 469 approximately one-third of nitrate in the river discharge [Sugimoto et al., 2011]. Therefore, the impact of riverine water on the  $\delta^{15}$ N value of the POM was not evaluated in this study. 470

As nitrogen fixation and atmospheric deposition decreased the  $\delta^{15}$ N value of the POM in 471 the offshore area, the contributions of these nitrogenous inputs were estimated. Assuming that 472 all  $\delta^{15}$ N in the POM originating from nitrogen fixation, atmospheric deposition, and ammonium 473 474 was -1‰ [Knapp et al., 2011; Minagawa and Wada, 1986; Wu et al., 2008] and that from deep-475 sea nitrate was 4‰ based on  $\delta^{15}$ N of the nitrate [*Mino et al.*, 2020; Sugimoto et al., 2009; 476 Yoshikawa et al., 2018], the deep-sea nitrate-originating POM comprised only one-third of the average POM (36%) in the offshore area during summer ( $\delta^{15}$ N is 0.8%). This estimated 477 478 contribution of the deep-sea nitrate-originating POM was possibly an overestimation because the contributions of the dissolved organic nitrogen (DON), whose  $\delta^{15}N$  was high, as well as that of 479 480 nitrate, were ignored [Knapp et al., 2011]. In other seasons, nitrate concentration was often 481 detectable; thus, the isotopic fraction during nitrate assimilation was not ignored, and the 482 contribution of deep-sea nitrate must be underestimated in our calculations.

The source of new nitrogen is more appropriately estimated based on the  $\delta^{15}$ N value of 483 484 the sinking POM [Casciotti et al., 2008]. At station ALOHA set off Hawaii, the annual mean 485 contribution of nitrogen fixation is 26-69% of the exported POM [Böttjer et al., 2017; Casciotti 486 et al., 2008; Dore et al., 2002]. At station S1 set in the western North Pacific, the annual contribution of nitrogen fixation is 7–14% [*Mino et al.*, 2020]. The  $\delta^{15}$ N of the POM in the 487 488 present study during summer was similar to that measured in the euphotic layer at station 489 ALOHA during summer [Casciotti et al., 2008], slightly lower than that at station S1 [Mino et 490 al., 2020], and higher than that in the western South Pacific Subtropical Gyre [Shiozaki et al., 491 2014]. Therefore, the contributions of nitrogen fixation and atmospheric deposition to the new 492 production in the Kuroshio area during summer and at station ALOHA are significant, but these 493 are not lower than those in the western South Pacific Subtropical Gyre, and are at least one-

494 quarter of the new production in this area. Considering the yearly lsmean of  $\delta^{15}$ N, the

495 contribution of deep-sea nitrate varies interannually. For example, its contribution was ~30%

lower than the average in 2013 in both the models, but the reason was not clear in this study.

497

498 **5.** Conclusion

This study reports the seasonal variations in the  $\delta^{13}$ C and  $\delta^{15}$ N values of the POM in the 499 500 euphotic layer based on the middle term (~10 years) repeated seasonal observations. The spatioseasonal variations in  $\delta^{13}$ C and  $\delta^{15}$ N of POM show that the primary production in the vicinity of 501 502 the Kuroshio is supported by many physical and biological processes, i.e., during the nitratesufficient and well-mixed conditions in winter, a low  $\delta^{15}$ N indicates the dominance of nitrate-503 504 originating primary production. When the stratification develops, and nitrate depletes in the spring, a high  $\delta^{15}$ N suggests that the isotopic fractionation of nitrate does not occur. In the 505 506 summer, diazotrophs and atmospheric deposition play important roles in the primary production 507 in the offshore area, while contribution from deep-sea nitrate remains active in the coastal area; 508 the contribution of riverine nitrogenous nutrient is considered significant, but cannot be evaluated based on  $\delta^{15}$ N. The deep-sea nitrate-originating production is considered to be limited 509 510 to one-third of primary production in the offshore area during summer. The carbon dynamics also exhibit spatio-seasonality. In the coastal area, the spring diatom blooms result in high  $\delta^{13}$ C-511 POM, whereas in the offshore, high  $\delta^{13}$ C-POM is formed in the summer. High  $\delta^{13}$ C-POM is also 512 513 present in low-salinity water in the coastal area, which may be caused by the horizontal 514 advection of the ashore water, and significantly affects the primary production in the coastal 515 area. Therefore, complex biogeochemical processes support the primary production in the 516 vicinity of the Kuroshio and the subsequent fishery production in the Pacific.

517	The yearly variation of the lsmean $\delta^{15}N$ indicates that the proportion of deep-sea nitrate
518	and nitrogen fixation or atmospheric deposition for the new production is not homogenous.
519	However, the yearly linear trends and the effect of the Kuroshio large mender are not clearly
520	observed because the Kuroshio large mender occurred after 2018; recent changes in the $\delta^{15}N$ and
521	$\delta^{13}$ C values of the POM are not sufficient to elucidate the effects. As the Kuroshio path shows a
522	large impact on the ecosystems in the Kuroshio [Hiraoka et al., 2019; Nakata et al., 2000],
523	further studies are necessary to understand the yearly variations and anthropogenic impact on the
524	primary production of the Kuroshio area.
525	

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537 Table 1. Descriptions of the least Akaike Information Criterion generalized linear models (AIC GLMs)

538 and their coefficients of determination  $(r^2)$ . The abbreviations are the same as those described in eq. 3.

	Least AIC models	$r^2$
Linear fun	ction	
$\delta^{13}C$	f(Y) + f(A):f(Sea) + f(A) + f(Sea) + T + Sal + log-chl + log-nit + K	0.443
$\delta^{15}N$	f(Y) + f(A):f(Sea) + T	0.309
Quartic fu	nction	
$\delta^{13}C$	f(Y) + f(Sea) + p(T) + p(Sal) + p(log-chl) + p(log-nit) + p(K)	0.596
$\delta^{15}N$	f(Y) + f(A) + f(Sea) + p(T) + p(Sal) + p(log-chl) + p(log-nit)	0.387
Linear fun	ction including diatom abundance (10 m depth only)	
$\delta^{13}C_{10 m}$	f(A) + f(Sea) + Sal + log-Chl + log-Nit + log-Diat + K	0.817
$\delta^{15}N_{10m}$	f(Y) + f(A): $f(Sea) + f(A) + f(Sea) + T + log-Chl + log-Nit + log-Diat$	0.659

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#### 730 Figure Captions

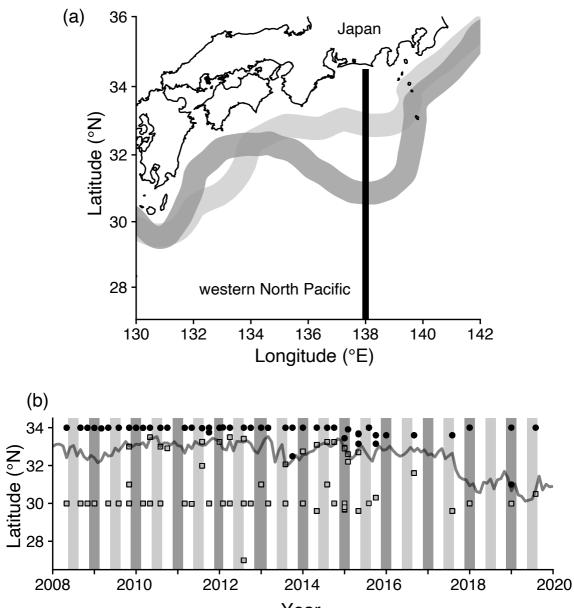
731 Figure 1. (a) Map of the sampling site and depiction of the flow of the Kuroshio current. Light 732 and dark gray lines represent the normal path (January 2015) and large-meandered 733 path of the Kuroshio current (January 2018), respectively. Black line depicts the "O-734 line". (b) Temporal variations of the sampling stations (circles and squares) and the 735 Kuroshio axis (solid gray line) along the O-line. Light and dark gray boxes in (b) 736 denote the summer and winter periods, respectively. Closed circles and squares 737 correspond to the coastal and offshore stations, respectively. 738 Figure 2. Seasonal variations in (a) temperature, (b) salinity, (c) chlorophyll a concentration, (d) 739 nitrate concentration, and (e) carbon-based diatom abundance. Dark blue and green 740 boxes indicate the data in the coastal and offshore areas, respectively. Chlorophyll a 741 concentration, nitrate concentration, and carbon-based diatom abundance are 742 converted into common-logarithm values. Each boxplot shows the medians (vertical 743 thick lines within boxes), upper and lower quartiles (boxes), quartile deviations 744 (horizontal bars), and outliers (circles). **Figure 3**. Scatter diagrams for  $\delta^{15}$ N and  $\delta^{13}$ C of POM along the O-line in the (a) coastal and (b) 745 746 offshore areas. Circles and crosses indicate the samples collected in the coastal and 747 offshore areas, and the colors denote the different seasons. The ellipses include 95% probability ellipse for each season and area. 748

# Figure 4. Relationship between δ<sup>13</sup>C and (a) temperature, (b) salinity, (c) common-logarithm transformed chlorophyll a concentration (log-chlorophyll), (d) common-logarithm transformed nitrate concentration (log-nitrate), (e) latitude of the Kuroshio axis, and (f) common-logarithm transformed carbon-based diatom abundance (log-diatom).

753	Diatom abundance data is available at only 10-m depth. The colors and shapes denote
754	the areas and seasons, respectively (blue: coastal; green: offshore; closed circle:
755	winter; open triangle: spring; cross: summer; closed square: autumn). The gray solid
756	dashed line with shadow represents the linear regression line and 95% confidence
757	values. When the linear regression line is the dashed line, the linear regression
758	relationship is not significant ( $p > 0.05$ ).
759	<b>Figure 5</b> . Relationship between $\delta^{15}$ N and (a) temperature, (b) salinity, (c) common-logarithm
760	transformed chlorophyll a concentration (log-chlorophyll), (d) common-logarithm
761	transformed nitrate concentration (log-nitrate), (e) latitude of the Kuroshio axis, and (f)
762	common-logarithm transformed carbon-based diatom abundance (log-diatom).
763	Diatom abundance data is available at only 10-m depth. The colors and shapes denote
764	the areas and seasons, respectively (blue: coastal; green: offshore; closed circle:
765	winter; open triangle: spring; cross: summer; closed square: autumn). The gray solid
766	dashed line with shadow represents the linear regression line and 95% confidence
767	values. When the linear regression line is the dashed line, the linear regression
768	relationship is not significant ( $p > 0.05$ ).
769	Figure 6. Responses of the least-AIC GLMs to $\delta^{13}$ C in terms of (a) temperature, (b) salinity, (c)
770	log-chlorophyll, (d) log-nitrate, (e) latitude of the Kuroshio axis, and (f) log-diatom.
771	Solid and dotted lines denote the GLMs of $\delta^{13}$ C fitting with the linear and quartic
772	functions, respectively. Blue and green vertical ticks on the x-axes indicate the data
773	points of the coastal and offshore areas, respectively. The numerical values of
774	common-log transformed values (c, d, and f) are re-transformed to the normal values,
775	but the axes are shown with the common-log transformed values.

776	Figure 7. Responses of the least-AIC GLMs to $\delta^{15}$ N in terms of (a) temperature, (b) salinity, (c)
777	log-chlorophyll, (d) log-nitrate, (e) latitude of the Kuroshio axis, and (f) log-diatom.
778	Solid and dotted lines denote the GLMs of $\delta^{15}$ N fitting with the linear and quartic
779	functions, respectively. Blue and green vertical ticks on the x-axes indicate the data
780	points of the coastal and offshore areas, respectively. The numerical values of
781	common-log transformed values (c, d, and f) are re-transformed to the normal values,
782	but the axes are shown with common-log transformed values. Gray horizontal lines
783	denote that the parameter is not selected in the least-AIC GLMs.
784	Figure 8. Yearly variations in the least square means (lsmean, closed circle) of $\delta^{13}C$ (a, b) and
784 785	<b>Figure 8</b> . Yearly variations in the least square means (Ismean, closed circle) of $\delta^{13}$ C (a, b) and $\delta^{15}$ N (c, d) based on the linear GLM (a, c) and quartic GLM (b, d). Vertical lines
785	$\delta^{15}$ N (c, d) based on the linear GLM (a, c) and quartic GLM (b, d). Vertical lines
785 786	$\delta^{15}$ N (c, d) based on the linear GLM (a, c) and quartic GLM (b, d). Vertical lines denote the 2 × standard errors ( <i>i.e.</i> , 95% confidence intervals).
785 786 787	<ul> <li>δ<sup>15</sup>N (c, d) based on the linear GLM (a, c) and quartic GLM (b, d). Vertical lines denote the 2 × standard errors (<i>i.e.</i>, 95% confidence intervals).</li> <li>Figure 9. Scatter plot of the calculated climatological δ<sup>13</sup>C and δ<sup>15</sup>N values in each season and</li> </ul>

Figure 1.



Year

Figure 2.

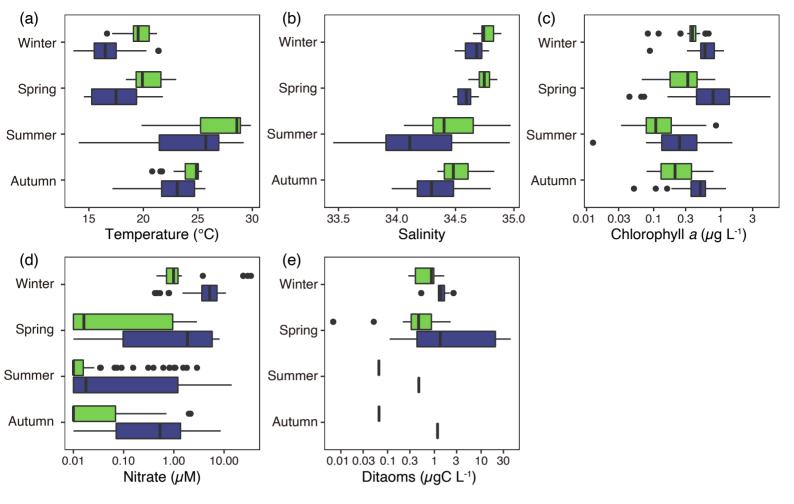


Figure 3.

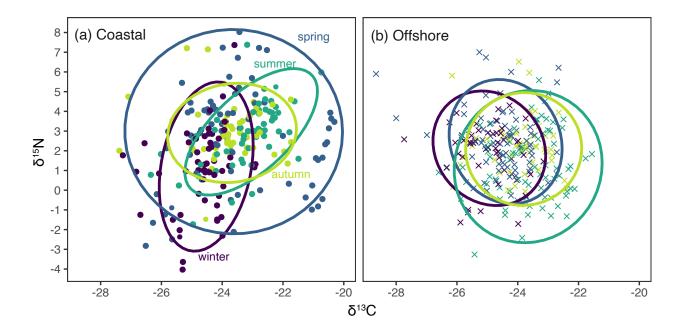


Figure 4.

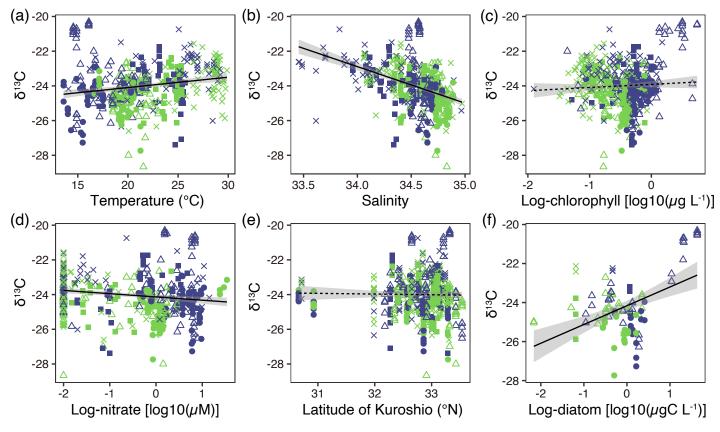


Figure 5.

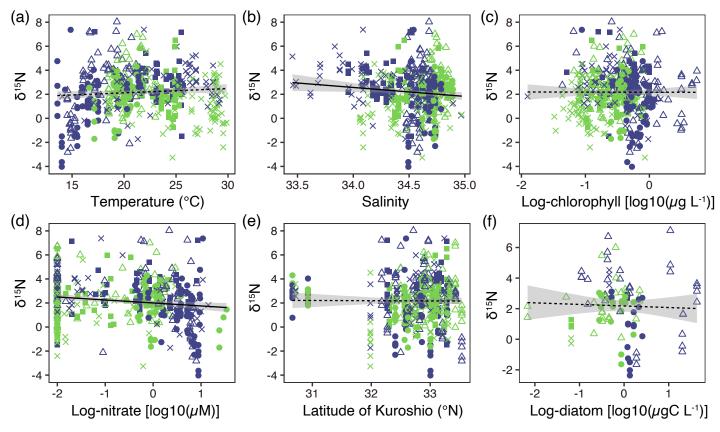


Figure 6.

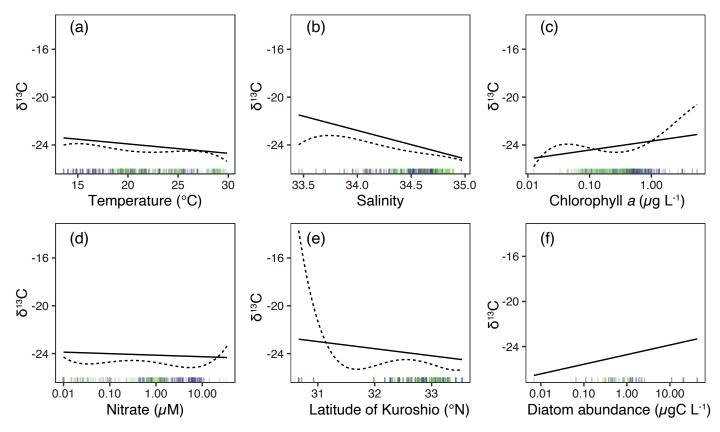


Figure 7.

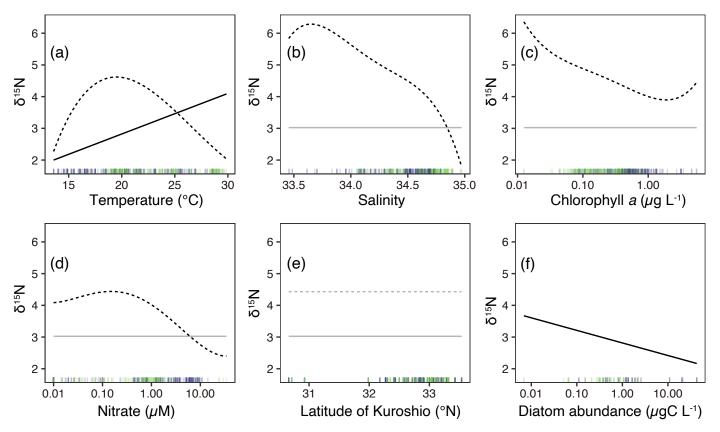
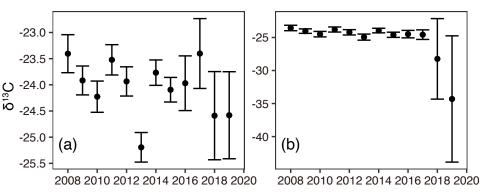


Figure 8.



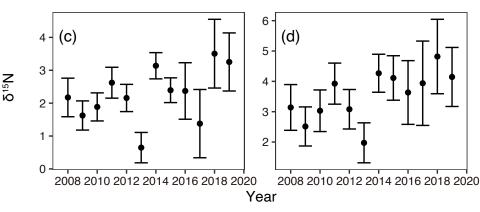


Figure 9.

