

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

**KODAMA Taketoshi^{1,*}; NISHIMOTO Atsushi²; HORII Sachiko³; ITO Daiki¹;
YAMAGUCHI Tamaha¹; HIDAKA Kiyotaka¹; SETOU Takashi¹; ONO Tsuneo¹**

¹Fisheries Resources Institute, Japan Fisheries Research and Education Agency

²Fisheries Technology Institute, Japan Fisheries Research and Education Agency

³Nagasaki Field Station, Fisheries Resources Institute, Japan Fisheries Research and Education
Agency

*Corresponding author

Address: 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan

e-mail: takekodama@affrc.go.jp

Running title: Stable isotope ratios of POM in the Kuroshio

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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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

Abstract

Stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ estimated based on the GLMs and seasonal median values of the environmental parameters was the highest in summer ($-22.4 \pm 0.2\text{‰}$ and $-22.9 \pm 0.2\text{‰}$ in the coastal and offshore areas, respectively) and lowest in winter in both areas ($-23.9 \pm 0.2\text{‰}$ and $-24.3 \pm 0.2\text{‰}$ in the coastal and offshore areas, respectively). Seasonal climatological $\delta^{15}\text{N}$ showed different spatial variations from spring to summer. The $\delta^{15}\text{N}$ value was the lowest during winter ($0.8 \pm 0.4\text{‰}$), and increased to a similar level during the other three seasons in the coastal area ($\sim 3\text{‰}$), suggesting that nitrate originating in the deep-sea water was the main source of new production from spring to autumn. In contrast, $\delta^{15}\text{N}$ in the offshore areas decreased from spring ($2.6 \pm 0.4\text{‰}$) to summer ($0.9 \pm 0.4\text{‰}$), suggesting 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.

Keywords: Particulate organic matter, Kuroshio, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, N_2 fixation, diatoms

1. Introduction

The ocean absorbs large amounts of carbon and nitrogen, which have continuously increased after the industrial revolution [Archer *et al.*, 2009; Duce *et al.*, 2008; Fowler *et al.*, 2013]. To detect the variations of the carbon and nitrogen dynamics in the ocean, the stable isotope ratios of carbon and nitrogen are useful tracers [Gruber *et al.*, 1999; Keeling, 1979; Sigman *et al.*, 2009; Sonnerup *et al.*, 1999]. The proportion of ^{13}C to ^{12}C in soluble carbon dioxide in seawater has linearly decreased at a rate of 0.2–0.1‰ decade⁻¹ with an increase in the carbon dioxide concentration in the atmosphere [Gruber *et al.*, 1999; Sonnerup *et al.*, 1999]. The long-term linear changes in the ratio of ^{15}N to ^{14}N have been rarely detected in the previous studies of the ocean [Chiba *et al.*, 2012; Christensen and Richardson, 2008], but the ratio of ^{15}N to ^{14}N in the coral skeleton-bound organic matter is decreasing with an increase in the anthropogenic nitrogen deposition in this century [Ren *et al.*, 2017].

The Kuroshio is the western boundary current of the North Pacific, and affects the long-term anthropogenic changes in the nitrogen and carbon dynamics [Ishii *et al.*, 2011; Watanabe *et al.*, 2005]. The dynamic also varies seasonally with the physical processes [Kodama *et al.*, 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 dinitrogen-fixation cyanobacteria and bacteria in the summer [Shiozaki *et al.*, 2018; Shiozaki *et al.*, 2015]. In addition, water is horizontally transported from the marginal seas and/or the coastal areas to the Kuroshio [Kodama *et al.*, 2015; Shiozaki *et al.*, 2015]. These complex carbon and nitrogen dynamics may support and affect biological productivity in the Kuroshio. The low trophic production in the Kuroshio is one of the most important keys of the fishery resources in Japan and the Pan-Pacific countries. The Kuroshio area is the spawning and nursery

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].

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

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

2. Materials and methods

2.1. Observation

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

The POM sampling was conducted seasonally from 2008 to 2015, and the observations were limited to the summer (August or September) and winter (January or February) after 2016 [Figure 1]. In summary, the observations were conducted at 23 stations in winter, 30 stations in spring (March, April, or May), 28 stations in summer, and 21 stations in autumn (October or November). After measuring the vertical distributions of the photosensitive available radiation (PAR) using a discrete PAR sensor (PRR-800 or INF-300, Biospherical Instruments Inc., San Diego, California, USA), the water samples were collected using the 12-L X-Niskin bottles attached directly to the cable at five different water depths for which the PAR values were 100, 50, 25, 10, and 1% relative to the surface until May 2016. The sampling depth was fixed after May 2016 within the upper 50 m. The vertical profiles of temperature and salinity were measured using a conductivity-temperature-depth (CTD) sensor (SBE9plus, Sea-Bird Scientific, Washington, USA). The discrete samples for determining nutrient and chlorophyll *a* concentrations were obtained using the Niskin bottles attached to the CTD-CMS (Carousel Multisampling System). Vertical resolution of the discrete samples (nutrients and chlorophyll *a* concentrations) was 10 m depth. As the environmental parameters determined using the POM samples were limited, the nearest depth data collected by the CTD-CMS was adopted for the following statistical analysis. The details of the nutrient and chlorophyll *a* analyses were previously reported in the literature [Kodama *et al.*, 2014b]. To treat the common-logarithm transformed values, the nitrate concentration below 0.01 μM was set as 0.01 μM .

Diatom abundance was investigated at a depth of 10 m for some stations. One liter of seawater samples were collected and fixed with glutaraldehyde solution. The carbon amount of the diatom cells was calculated based on the lengths of the major axes and shapes [Menden-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.

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 decarboxylation.

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

$$\delta X = (R_{\text{sample}} / R_{\text{standard}} - 1.0) \times 1000, \quad (1)$$

where X, R_{sample} , and R_{standard} are the target elements (C or N), and the heavy (^{13}C and ^{15}N) to light (^{12}C and ^{14}N) isotope ratios of the sample and standard, respectively. The standards included atmospheric N_2 for nitrogen and Vienna Pee Dee Belemnite for carbon. The precision

of the analysis varied with the amounts of carbon and nitrogen and between the spectrometers, but the values were within 0.2‰ for $\delta^{13}\text{C}$ and 1‰ for $\delta^{15}\text{N}$. Considering the quality of the standards, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each sample were rounded off to one decimal place.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM were sometimes the outlier values. Therefore, the mean and standard deviations (sd) were calculated for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all the samples, and the samples whose $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values differed by $>3 \times \text{sd}$ from the mean values were eliminated. This quality control process was performed twice. In addition, the samples for which the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were available, but the environmental parameters were not recorded were removed. As a result, 490 samples out of 526 samples were used in the following statistical analyses. The breakdown of seasonality was as follows: the numbers in winter, spring, summer, and autumn were 108, 147, 133, and 102, respectively. Our data was in the supplemental information.

2.3. Statistical Analysis

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

$$\delta X \sim N(\mu, \sigma^2) \quad (2)$$

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

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

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

$$\delta X \sim \text{glm}[f(Y) + f(A):f(\text{Sea}) + f(A) + f(\text{Sea}) + p(T, 4) + p(\text{Sal}, 4) + p(\log\text{-Chl}, 4) + p(\log\text{-Nit}, 4) + p(\text{Dep}, 4) + p(K, 4)] \quad (5)$$

Here, $p(Z, 4)$ denotes the numerical explanatory variables (Z) applied as quartic functions (degree of 4). Quartic function was selected to avoid overfitting. Hereafter, the model described by eq. 5 is named as the quartic function GLM.

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

3. Results

3.1. Seasonality of environmental parameters

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

226

227 3.2. Isotope ratios

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

241 Significant (F -test, $p < 0.05$) linear relationships between $\delta^{13}\text{C}$ and several environmental
242 factors, *i.e.* temperature, salinity, and log-nitrate were observed [Figure 4]. The coefficients and
243 intercept of the linear regression between $\delta^{13}\text{C}$ and temperature were 0.060 ± 0.013 (‰°C⁻¹) and
244 -25.3 ± 0.3 ‰, respectively, and those between $\delta^{13}\text{C}$ and salinity were -2.1 ± 0.20 (‰) and $48.7 \pm$
245 6.6 ‰, respectively. The coefficient of determination (r^2) shows salinity was the strongest
246 explanatory parameter ($r^2 = 0.197$), and temperature and log-nitrate were weak parameters ($r^2 =$
247 0.037 and 0.026 , respectively). There was no significant relationship between $\delta^{13}\text{C}$ and log-

chlorophyll ($p > 0.2$), and the latitude of the Kuroshio axis [Figure 4]. There was a positive relationship between $\delta^{13}\text{C}$ and log-diatom [Figure 4].

Significant (F -test, $p < 0.05$) linear relationships were observed between $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ and other environmental factors such as temperature, log-chlorophyll, latitude of Kuroshio axis, and log-diatom. The $\delta^{15}\text{N}$ 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].

3.4. Results of GLMs

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

The responses of the numerical explanatory variables in the least-AIC GLMs are shown in Figure 6. In the full data set model using only the linear functions for $\delta^{13}\text{C}$, the negative effects to $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$, the effect of the latitude of the Kuroshio axis was different from that with the linear function: $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ at 10 m depth showed that log-diatom significantly and positively affected the $\delta^{13}\text{C}$ values [Figure 6].

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

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

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

For the calculation of the seasonal and spatial lsmean values, the environmental parameters except season and area were fixed. However, in the observations, the environmental parameters showed some degrees of collinearity to the season and/or areas. Therefore, the seasonal and spatial “climatological” $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calculated using the least-AIC GLMs and median values of the environmental parameters of each season and area. The year was fixed as 2012, when both the lsmean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were neither maximum nor minimum. As a result, significant spatial difference in the seasonality of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was 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 function GLM [Table I], the numerical values hereafter correspond to the results of the quartic function GLM. In the coastal area, both the climatological $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were the lowest in winter ($-23.9 \pm 0.2\text{‰}$ [mean \pm SE] and $0.8 \pm 0.4\text{‰}$, respectively). The $\delta^{15}\text{N}$ values were higher than 2.5‰ and similar in the other three seasons (2.7 ± 0.4 , 2.7 ± 0.4 and $3.2 \pm 0.4\text{‰}$ in spring, summer, and autumn, respectively). The $\delta^{13}\text{C}$ values were highest in summer ($-22.4 \pm 0.2\text{‰}$), and those during spring and autumn were similar (-23.1 ± 0.2 and -23.2 ± 0.2 , respectively). In the offshore area, the seasonal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varied in a narrower range

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

4. Discussion

4.1. Comparison of the previous studies

In the upstream of the Kuroshio and the East China Sea, the horizontal gradients of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the POM were reported [Wu *et al.*, 2003] to range from -19 to -31‰ for $\delta^{13}\text{C}$ and from 0.7 to 9.5‰ for $\delta^{15}\text{N}$ in autumn. Saino and Hattori [1987] reported only the $\delta^{15}\text{N}$ value in 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 $\delta^{13}\text{C}$ and between ~1‰ and ~4‰ for $\delta^{15}\text{N}$ in the East China Sea during summer. Takai *et al.* [2007] reported the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM ranging from -24.2 to -19.1 and 0.7 to 6.8, respectively, around the 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 Pacific Subtropical Gyre. In their observations, the $\delta^{15}\text{N}$ values of the POM ranged between 1.5 and 2.6‰ at 0–100 m depth [Mino *et al.*, 2020], but Mino *et al.* [2020] did not report the $\delta^{13}\text{C}$ values. The ranges for these values are consistent with the results presented herein. Therefore, considering that results in the present study are based on the over-decadal seasonal repeated observations, the climatological $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values herein [Figure 9] can be treated as the

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

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

4.2. Carbon dynamics in the vicinity of the Kuroshio

Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM are different from those of the phytoplankton because POM contains heterotrophic small planktons and detritus, but it is considered that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of POM are largely dependent on those of the phytoplankton in open water. The $\delta^{13}\text{C}$ value of the phytoplankton depends on the CO_2 concentration in water, growth rate, and cell geometry of each species [Popp *et al.*, 1998]. The growth rate of the phytoplankton depends on temperature, nutrient concentration, and light conditions [Cross *et al.*, 2015], and CO_2 concentration in water depends on the temperature, atmospheric CO_2 concentration, and phytoplankton growth. As the result, the $\delta^{13}\text{C}$ value of the POM or phytoplankton becomes

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 $\delta^{13}\text{C}$ response to temperature was weak or negligible in the present study contrary to that observed in the previous studies [Figures 4 and 6]. The coefficient of simple linear regression analysis between temperature and $\delta^{13}\text{C}$ [Figure 4] was 0.06 ± 0.01 ($\text{‰ } ^\circ\text{C}^{-1}$), which was smaller than that obtained by the global data estimation (0.17 ± 0.04) [Goericke and Fry, 1994] and in western North Pacific (0.25) [Wada et al., 2012]. The seasonal variations indicated that the $\delta^{13}\text{C}$ value of the POM was high in warm water (for example, $\delta^{13}\text{C}$ in the offshore area during summer), but the other factors such as salinity and chlorophyll *a* concentration more significantly affected the $\delta^{13}\text{C}$ variations in the Kuroshio. The other possible mechanism for the weak or negligible temperature response was the gravitational downward flux 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 $\delta^{13}\text{C}$ and *in situ* temperature was weaker than the empirical values, because the high $\delta^{13}\text{C}$ of the POM produced in the warm surface layer was mixed with the low $\delta^{13}\text{C}$ of POM produced in the cold subsurface layer.

The highest $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ value of the POM in the coastal area was higher than

that in the offshore. The positive coefficient of log-chlorophyll for $\delta^{13}\text{C}$ in the GLM also supported that $\delta^{13}\text{C}$ of POM elevates during the rapid phytoplankton growth. These observations suggested that carbon sequestration during spring in the coastal area was active, and $\delta^{13}\text{C}$ of POM varied with the phytoplankton community structure in the real ocean.

The salinity decreased during summer and autumn in the coastal area and showed a negative effect on $\delta^{13}\text{C}$ [Figures 4 and 6]. Thus, a high $\delta^{13}\text{C}$ was observed in less-saline water supply. In the estuary, the $\delta^{13}\text{C}$ value of the POM is sometimes affected by the riverine POM [Bănară et al., 2014]. The intercept of the simple linear relationship between salinity and $\delta^{13}\text{C}$ indicated that the $\delta^{13}\text{C}$ value of the POM in original freshwater was $48.7 \pm 6.6\text{‰}$ if the relationship between salinity and $\delta^{13}\text{C}$ was established only based on the results of mixing riverine and oceanic POM. This estimated value was unrealistic: the $\delta^{13}\text{C}$ of the riverine POM 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., 2004], and $-26.9 \pm 1.1\text{‰}$ in the Tokyo bay [Ogawa and Ogura, 1997]. Therefore, the high $\delta^{13}\text{C}$ of the POM in the summer was not the result of the horizontal advective transport of the riverine POM.

In addition to the POM, inorganic nutrients are also supplied by the riverine freshwater. With these nutrients, the high $\delta^{13}\text{C}$ 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- $\delta^{13}\text{C}$ POM is possibly advected from the estuary or eutrophic ashore areas to the investigated area in the present study. In fact, a high $\delta^{13}\text{C}$ was observed in the ashore area near this investigated area

by *Takai et al.* [2007]. Therefore, considering the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ value of the POM in the GLMs [*Figure 6*]. The coefficients of the linear function GLM suggested that when the Kuroshio flowed near the coast, the $\delta^{13}\text{C}$ value of the POM decreased, and when the Kuroshio large meander occurred, the $\delta^{13}\text{C}$ value of the POM increased. The quartic function GLM indicated that the $\delta^{13}\text{C}$ value of the POM was significantly higher when the Kuroshio large meander occurred, but considering the yearly lsmean, the response to the Kuroshio path in the quartic function GLM was unreliable and overfitted. Additional studies are necessary to understand the relationship between the Kuroshio path and $\delta^{13}\text{C}$.

4.3. Nitrogen dynamics in the vicinity of the Kuroshio

The $\delta^{15}\text{N}$ value of the POM varies with $\delta^{15}\text{N}$ of the original nitrogen sources (nitrate, ammonium, and N_2 fixation), and degree of isotopic fractionation. The rate of isotopic fractionation of the phytoplankton is controlled by its growth rate [*Wada and Hattori, 1990*]. The highest $\delta^{15}\text{N}$ value of the POM is produced under nitrate-depleted condition, in which the nitrogen isotopic fractionation of nitrate does not occur [*Wada et al., 2012*]. The lowest $\delta^{15}\text{N}$ value of the phytoplankton originates from nitrogen fixation [*Minagawa and Wada, 1986*]. The $\delta^{15}\text{N}$ value of the nitrate is 3–5‰ in the vicinity of the Kuroshio [*Sugimoto et al., 2009; Yoshikawa et al., 2018*]. In winter, vertical mixing occurs, and nitrate is present at a micromolar level both in the coastal and offshore areas in the euphotic layer [*Figure 2*]. In spring, stratification starts occurring, and the nitrate in the offshore depletes but is detectable at the sub-

micromolar level [Figure 2]. The $\delta^{15}\text{N}$ value of the POM (phytoplankton) is lower than that of the nitrate under conditions with sufficient nitrate [Fogel and Cifuentes, 1993; Montoya and McCarthy, 1995; Wada, 1980]. Therefore, the lower climatological $\delta^{15}\text{N}$ values of the POM in winter (0.8 ± 0.4 and $1.6 \pm 0.4\text{‰}$ in the coastal and offshore areas, respectively, Figure 9) compared to those during spring were owing to the difference in the nitrate, *i.e.*, nitrate consumption was lower than nitrate supply in the winter, while nitrate consumption was greater than nitrate supply in the spring. Similar phenomenon was reported by Mino *et al.* [2020]. The $\delta^{15}\text{N}$ value of the POM in the sediment traps [Mino *et al.*, 2020] was higher than the values in water, but a decrease in the $\delta^{15}\text{N}$ value of the POM in the winter was similar because of the 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 nitrate-originating new production was active in the coastal waters, but not in the offshore water.

Two possible sources that decrease $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of *Trichodesmium*

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

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

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

As nitrogen fixation and atmospheric deposition decreased the $\delta^{15}\text{N}$ value of the POM in the offshore area, the contributions of these nitrogenous inputs were estimated. Assuming that all $\delta^{15}\text{N}$ in the POM originating from nitrogen fixation, atmospheric deposition, and ammonium was ‰ [Knapp *et al.*, 2011; Minagawa and Wada, 1986; Wu *et al.*, 2008] and that from deep-sea nitrate was ‰ based on $\delta^{15}\text{N}$ of the nitrate [Mino *et al.*, 2020; Sugimoto *et al.*, 2009; 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 ($\delta^{15}\text{N}$ is ‰). This estimated contribution of the deep-sea nitrate-originating POM was possibly an overestimation because the contributions of the dissolved organic nitrogen (DON), whose $\delta^{15}\text{N}$ was high, as well as that of nitrate, were ignored [Knapp *et al.*, 2011]. In other seasons, nitrate concentration was often detectable; thus, the isotopic fraction during nitrate assimilation was not ignored, and the contribution of deep-sea nitrate must be underestimated in our calculations.

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

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

5. Conclusion

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

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

Acknowledgement

We thank the captains, officers, crew members, and scientists of the R/V *Soyo-maru* cruise for their cooperation at the sea. We are grateful to K. Yamada and T. Ichikawa for analyses and providing data. E. Wada and S. Ohshimo kindly commented on an earlier draft of the manuscript. Our data is published as the supplemental information of this article. This study was financially supported by Fisheries Agency, Japan Fisheries Research and Education Agency, Agriculture, Forestry and Fisheries Research Council through a research project entitled “Development of Technologies for Mitigation and Adaptation to Climate Change in Agriculture, Forestry and Fisheries”, and JSPS KAKENHI Grant Numbers 19K06198 (to TK), 19H03038 (to KH, TY, and TI), and 19K24396 (to TY).

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 function | | |
| $\delta^{13}\text{C}$ | $f(Y) + f(A):f(\text{Sea}) + f(A) + f(\text{Sea}) + T + \text{Sal} + \log\text{-chl} + \log\text{-nit} + K$ | 0.443 |
| $\delta^{15}\text{N}$ | $f(Y) + f(A):f(\text{Sea}) + T$ | 0.309 |
| Quartic function | | |
| $\delta^{13}\text{C}$ | $f(Y) + f(\text{Sea}) + p(T) + p(\text{Sal}) + p(\log\text{-chl}) + p(\log\text{-nit}) + p(K)$ | 0.596 |
| $\delta^{15}\text{N}$ | $f(Y) + f(A) + f(\text{Sea}) + p(T) + p(\text{Sal}) + p(\log\text{-chl}) + p(\log\text{-nit})$ | 0.387 |
| Linear function including diatom abundance (10 m depth only) | | |
| $\delta^{13}\text{C}_{10\text{ m}}$ | $f(A) + f(\text{Sea}) + \text{Sal} + \log\text{-Chl} + \log\text{-Nit} + \log\text{-Diat} + K$ | 0.817 |
| $\delta^{15}\text{N}_{10\text{ m}}$ | $f(Y) + f(A):f(\text{Sea}) + f(A) + f(\text{Sea}) + T + \log\text{-Chl} + \log\text{-Nit} + \log\text{-Diat}$ | 0.659 |

539

540 **References**

- 541 Aita, M. N., K. Tadokoro, N. O. Ogawa, F. Hyodo, R. Ishii, S. L. Smith, T. Saino, M. J. Kishi, S.-I.
542 Saitoh, and E. Wada (2011), Linear relationship between carbon and nitrogen isotope ratios along
543 simple food chains in marine environments, *J. Plankton Res.*, 33(11), 1629-1642,
544 doi:10.1093/plankt/fbr070.
- 545 Archer, D., et al. (2009), Atmospheric lifetime of fossil fuel carbon dioxide, *Annu. Rev. Earth Planet. Sci.*,
546 37(1), 117-134, doi:10.1146/annurev.earth.031208.100206.
- 547 Bănar, D., F. Carlotti, A. Barani, G. Grégori, N. Neffati, and M. Harmelin-Vivien (2014), Seasonal
548 variation of stable isotope ratios of size-fractionated zooplankton in the Bay of Marseille (NW
549 Mediterranean Sea), *J. Plankton Res.*, 36(1), 145-156, doi:10.1093/plankt/fbt083.
- 550 Böttjer, D., J. E. Dore, D. M. Karl, R. M. Letelier, C. Mahaffey, S. T. Wilson, J. Zehr, and M. J. Church
551 (2017), Temporal variability of nitrogen fixation and particulate nitrogen export at Station
552 ALOHA, *Limnol. Oceanogr.*, 62(1), 200-216, doi:10.1002/lno.10386.
- 553 Casciotti, K. L., T. W. Trull, D. M. Glover, and D. Davies (2008), Constraints on nitrogen cycling at the
554 subtropical North Pacific Station ALOHA from isotopic measurements of nitrate and particulate
555 nitrogen, *Deep Sea Res. II*, 55(14), 1661-1672, doi:10.1016/j.dsr2.2008.04.017.
- 556 Chiba, S., H. Sugisaki, A. Kuwata, K. Tadokoro, T. Kobari, A. Yamaguchi, and D. L. Mackas (2012), Pan-
557 North Pacific comparison of long-term variation in Neocalanus copepods based on stable isotope
558 analysis, *Prog. Oceanogr.*, 97-100, 63-75, doi:10.1016/j.pocean.2011.11.007.
- 559 Christensen, J. T., and K. Richardson (2008), Stable isotope evidence of long-term changes in the North
560 Sea food web structure, *Mar. Ecol. Prog. Ser.*, 368, 1-8, doi:10.3354/meps07635.
- 561 Cross, W. F., J. M. Hood, J. P. Benstead, A. D. Huryn, and D. Nelson (2015), Interactions between
562 temperature and nutrients across levels of ecological organization, *Glob Chang Biol*, 21(3), 1025-
563 1040, doi:10.1111/gcb.12809.
- 564 Dore, J. E., J. R. Brum, L. M. Tupas, and D. M. Karl (2002), Seasonal and interannual variability in
565 sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean,
566 *Limnol. Oceanogr.*, 47(6), 1595-1607, doi:10.4319/lo.2002.47.6.1595.
- 567 Duce, R. A., et al. (2008), Impacts of atmospheric anthropogenic nitrogen on the open ocean, *Science*,
568 320(5878), 893-897, doi:10.1126/science.1150369.
- 569 Fogel, M. L., and L. A. Cifuentes (1993), Isotope fractionation during primary production, in *Org.*
570 *Geochem.*, edited by M. H. Engel and S. A. Macko, pp. 73-98, Springer US, Boston, MA,
571 doi:10.1007/978-1-4615-2890-6_3.
- 572 Fowler, D., M. Coyle, U. Skiba, M. A. Sutton, J. N. Cape, S. Reis, L. J. Sheppard, A. Jenkins, B.
573 Grizzetti, and J. N. Galloway (2013), The global nitrogen cycle in the twenty-first century, *Philos.*
574 *T. R. Soc. B*, 368(1621), 20130164, doi:10.1098/rstb.2013.0164.

575 Fox, J., and S. Weisberg (2018), *An R companion to applied regression*, Sage publications.

576 Goericke, R., and B. Fry (1994), Variations of marine plankton $\delta^{13}\text{C}$ with latitude, temperature, and
577 dissolved CO_2 in the world ocean, *Global Biogeochem. Cycles*, 8(1), 85-90,
578 doi:10.1029/93gb03272.

579 Gruber, N., C. D. Keeling, R. B. Bacastow, P. R. Guenther, T. J. Lueker, M. Wahlen, H. A. J. Meijer, W.
580 G. Mook, and T. F. Stocker (1999), Spatiotemporal patterns of carbon-13 in the global surface
581 oceans and the oceanic suess effect, *Global Biogeochem. Cycles*, 13(2), 307-335,
582 doi:10.1029/1999gb900019.

583 Hiraoka, Y., K. Fujioka, H. Fukuda, M. Watai, and S. Ohshimo (2019), Interannual variation of the diet
584 shifts and their effects on the fatness and growth of age-0 Pacific bluefin tuna (*Thunnus*
585 *orientalis*) off the southwestern Pacific coast of Japan, *Fish. Oceanogr.*, 28(4), 419-433,
586 doi:10.1111/fog.12421.

587 Horii, S., K. Takahashi, T. Shiozaki, F. Hashihama, and K. Furuya (2018), Stable isotopic evidence for the
588 differential contribution of diazotrophs to the epipelagic grazing food chain in the mid-Pacific
589 Ocean, *Global Ecol. Biogeogr.*, 27(12), 1467-1480, doi:10.1111/geb.12823.

590 Ishii, M., N. Kosugi, D. Sasano, S. Saito, T. Midorikawa, and H. Y. Inoue (2011), Ocean acidification off
591 the south coast of Japan: A result from time series observations of CO_2 parameters from 1994 to
592 2008, *J. Geophys. Res.*, 116(C6), doi:10.1029/2010jc006831.

593 Keeling, C. D. (1979), The Suess effect: ^{13}C Carbon- ^{14}C Carbon interrelations, *Environ. Int.*, 2(4-6), 229-
594 300, doi:10.1016/0160-4120(79)90005-9.

595 Knapp, A. N., D. M. Sigman, F. Lipschultz, A. B. Kustka, and D. G. Capone (2011), Interbasin isotopic
596 correspondence between upper-ocean bulk DON and subsurface nitrate and its implications for
597 marine nitrogen cycling, *Global Biogeochem. Cycles*, 25(4), doi:10.1029/2010gb003878.

598 Kodama, T., K. Furuya, F. Hashihama, S. Takeda, and J. Kanda (2011), Occurrence of rain-origin nitrate
599 patches at the nutrient-depleted surface in the East China Sea and the Philippine Sea during
600 summer, *J. Geophys. Res.*, 116(C8), C08003, doi:10.1029/2010jc006814.

601 Kodama, T., T. Ichikawa, K. Hidaka, and K. Furuya (2014a), A highly sensitive and large concentration
602 range colorimetric continuous flow analysis for ammonium concentration, *J. Oceanogr.*, 71(1),
603 65-75, doi:10.1007/s10872-014-0260-6.

604 Kodama, T., T. Setou, M. Masujima, M. Okazaki, and T. Ichikawa (2015), Intrusions of excess nitrate in
605 the Kuroshio subsurface layer, *Cont. Shelf Res.*, 110, 191-200, doi:10.1016/j.csr.2015.10.012.

606 Kodama, T., Y. Shimizu, T. Ichikawa, Y. Hiroe, A. Kusaka, H. Morita, M. Shimizu, and K. Hidaka
607 (2014b), Seasonal and spatial contrast in the surface layer nutrient content around the Kuroshio
608 along 138°E , observed between 2002 and 2013, *J. Oceanogr.*, 70(6), 489-503,
609 doi:10.1007/s10872-014-0245-5.

610 Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt, and S. A. Macko (1995), Dependence of
611 phytoplankton carbon isotopic composition on growth rate and $[\text{CO}_2]_{\text{aq}}$: Theoretical

612 considerations and experimental results, *Geochim. Cosmochim. Acta*, 59(6), 1131-1138,
613 doi:10.1016/0016-7037(95)00030-4.

614 Lenth, R. (2020), emmeans: Estimated marginal means, aka least-squares means, edited, [https://CRAN.R-](https://CRAN.R-project.org/package=emmean)
615 [project.org/package=emmean](https://CRAN.R-project.org/package=emmean).

616 Lorrain, A., et al. (2020), Trends in tuna carbon isotopes suggest global changes in pelagic phytoplankton
617 communities, *Glob Chang Biol*, 26(2), 458-470, doi:10.1111/gcb.14858.

618 Menden-Deuer, S., and E. J. Lessard (2000), Carbon to volume relationships for dinoflagellates, diatoms,
619 and other protist plankton, *Limnol. Oceanogr.*, 45(3), 569-579, doi:10.4319/lo.2000.45.3.0569.

620 Minagawa, M., M. Ohashi, T. Kuramoto, and N. Noda (2001), $\delta^{15}\text{N}$ of PON and nitrate as a clue to the
621 origin and transformation of nitrogen in the subarctic North Pacific and its marginal sea, *J.*
622 *Oceanogr.*, 57(3), 285-300, doi:10.1023/a:1012430512137.

623 Minagawa, M., and E. Wada (1986), Nitrogen isotope ratios of red tide organisms in the East China Sea:
624 A characterization of biological nitrogen fixation, *Mar. Chem.*, 19(3), 245-259, doi:10.1016/0304-
625 4203(86)90026-5.

626 Mino, Y., et al. (2020), Seasonal and Interannual Variations in Nitrogen Availability and Particle Export in
627 the Northwestern North Pacific Subtropical Gyre, *J. Geophys. Res.*, 125(5), e2019JC015600,
628 doi:10.1029/2019jc015600.

629 Montoya, J. P., and J. J. McCarthy (1995), Isotopic fractionation during nitrate uptake by phytoplankton
630 grown in continuous culture, *J. Plankton Res.*, 17(3), 439-464, doi:10.1093/plankt/17.3.439.

631 Nagao, S., T. Aramaki, N. Fujitake, H. Kodama, T. Tanaka, S. Ochiai, M. Uchida, Y. Shibata, and M.
632 Yamamoto (2013), Variation of $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values of dissolved humic and fulvic acids in the
633 Tokachi river system in northern Japan, *Radiocarbon*, 55(Nr2-3), 1007-1016.

634 Nagao, S., T. Aramaki, O. Seki, M. Uchida, and Y. Shibata (2010), Carbon isotopes and lignin
635 composition of POC in a small river in Bekanbeushi Moor, northern Japan, *Nuclear Instruments*
636 *and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 268(7-
637 8), 1098-1101, doi:10.1016/j.nimb.2009.10.108.

638 Nakata, H., S. Funakoshi, and M. Nakamura (2000), Alternating dominance of postlarval sardine and
639 anchovy caught by coastal fishery in relation to the Kuroshio meander in the Enshu-nada Sea,
640 *Fish. Oceanogr.*, 9(3), 248-258, doi:10.1046/j.1365-2419.2000.00140.x.

641 Ogawa, N., and N. Ogura (1997), Dynamics of particulate organic matter in the Tamagawa estuary and
642 inner Tokyo Bay, *Estuar. Coast. Shelf. Sci.*, 44(3), 263-273, doi:10.1006/ecss.1996.0118.

643 Ohshimo, S., D. J. Madigan, T. Kodama, H. Tanaka, K. Komoto, S. Suyama, T. Ono, and T. Yamakawa
644 (2019), Isoscapes reveal patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of pelagic forage fish and squid in the
645 Northwest Pacific Ocean, *Prog. Oceanogr.*, 175, 124-138, doi:10.1016/j.pocean.2019.04.003.

646 Oka, E., M. Ishii, T. Nakano, T. Suga, S. Kouketsu, M. Miyamoto, H. Nakano, B. Qiu, S. Sugimoto, and
647 Y. Takatani (2018), Fifty years of the 137°E repeat hydrographic section in the western North
648 Pacific Ocean, *J. Oceanogr.*, 74(2), 115-145, doi:10.1007/s10872-017-0461-x.

649 Oka, E., K. Yamada, D. Sasano, K. Enyo, T. Nakano, and M. Ishii (2019), Remotely forced decadal
650 physical and biogeochemical variability of North Pacific Subtropical Mode water over the last 40
651 years, *Geophys. Res. Lett.*, 46(3), 1555-1561, doi:10.1029/2018gl081330.

652 Popp, B. N., E. A. Laws, R. R. Bidigare, J. E. Dore, K. L. Hanson, and S. G. Wakeham (1998), Effect of
653 phytoplankton cell geometry on carbon isotopic fractionation, *Geochim. Cosmochim. Acta*, 62(1),
654 69-77, doi:10.1016/s0016-7037(97)00333-5.

655 R Core Team (2020), R: A language and environment for statistical computing, edited by R Foundation
656 for Statistical Computing, <https://www.R-project.org/>. Vienna, Austria.

657 Ren, H., Y. C. Chen, X. T. Wang, G. T. F. Wong, A. L. Cohen, T. M. DeCarlo, M. A. Weigand, H. S. Mii,
658 and D. M. Sigman (2017), 21st-century rise in anthropogenic nitrogen deposition on a remote
659 coral reef, *Science*, 356(6339), 749-752, doi:10.1126/science.aal3869.

660 Saino, T., and A. Hattori (1987), Geographical variation of the water column distribution of suspended
661 particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its marginal seas,
662 *Deep Sea Res. A*, 34(5-6), 807-827, doi:10.1016/0198-0149(87)90038-0.

663 Shiozaki, T., K. Furuya, T. Kodama, S. Kitajima, S. Takeda, T. Takemura, and J. Kanda (2010), New
664 estimation of N_2 fixation in the western and central Pacific Ocean and its marginal seas, *Global*
665 *Biogeochem. Cycles*, 24(1), GB1015, doi:10.1029/2009gb003620.

666 Shiozaki, T., T. Kodama, and K. Furuya (2014), Large-scale impact of the island mass effect through
667 nitrogen fixation in the western South Pacific Ocean, *Geophys. Res. Lett.*, 41(8), 2907-2913,
668 doi:10.1002/2014GL059835.

669 Shiozaki, T., Y. Kondo, D. Yuasa, and S. Takeda (2018), Distribution of major diazotrophs in the surface
670 water of the Kuroshio from northeastern Taiwan to south of mainland Japan, *J. Plankton Res.*,
671 40(4), 407-419, doi:10.1093/plankt/fby027.

672 Shiozaki, T., S. Takeda, S. Itoh, T. Kodama, X. Liu, F. Hashihama, and K. Furuya (2015), Why is
673 *Trichodesmium* abundant in the Kuroshio?, *Biogeosciences*, 12(23), 6931-6943, doi:10.5194/bg-
674 12-6931-2015.

675 Sigman, D. M., K. L. Karsh, and K. L. Casciotti (2009), Nitrogen isotopes in the ocean, in *Encyclopedia*
676 *of Ocean Sciences*, edited by J. H. Steele, pp. 40-54, Academic Press, Oxford, doi:10.1016/b978-
677 012374473-9.00632-9.

678 Sonnerup, R. E., P. D. Quay, A. P. McNichol, J. L. Bullister, T. A. Westby, and H. L. Anderson (1999),
679 Reconstructing the oceanic ^{13}C Suess Effect, *Global Biogeochem. Cycles*, 13(4), 857-872,
680 doi:10.1029/1999gb900027.

681 Sugimoto, R., A. Kasai, K. Fujita, K. Sakaguchi, and T. Mizuno (2011), Assessment of nitrogen loading
682 from the Kiso-Sansen Rivers into Ise Bay using stable isotopes, *J. Oceanogr.*, 67(2), 231-240,
683 doi:10.1007/s10872-011-0022-7.

684 Sugimoto, R., A. Kasai, T. Miyajima, and K. Fujita (2009), Nitrogen isotope ratios of nitrate as a clue to
685 the origin of nitrogen on the Pacific coast of Japan, *Cont. Shelf Res.*, 29(10), 1303-1309,
686 doi:10.1016/j.csr.2009.03.007.

687 Sugimoto, R., A. Kasai, S. Yamao, T. Fujiwara, and T. Kimura (2004), Variation in particulate organic
688 matter accompanying changes of river discharge in Ise Bay, *Bull. Jap. Soc. Fish. Oceanogr.*,
689 68(3), 142-150.

690 Takai, N., N. Hirose, T. Osawa, K. Hagiwara, T. Kojima, Y. Okazaki, T. Kuwae, T. Taniuchi, and K.
691 Yoshihara (2007), Carbon source and trophic position of pelagic fish in coastal waters of south-
692 eastern Izu Peninsula, Japan, identified by stable isotope analysis, *Fish. Sci.*, 73(3), 593-608,
693 doi:10.1111/j.1444-2906.2007.01372.x.

694 Tanaka, Y., A. Tawa, T. Ishihara, E. Sawai, M. Nakae, M. Masujima, and T. Kodama (2019), Occurrence
695 of Pacific bluefin tuna *Thunnus orientalis* larvae off the Pacific coast of Tohoku area,
696 northeastern Japan: Possibility of the discovery of the third spawning ground, *Fish. Oceanogr.*,
697 29(1), 46-51, doi:10.1111/fog.12445.

698 Tawa, A., T. Kodama, K. Sakuma, T. Ishihara, and S. Ohshimo (2020), Fine-scale horizontal distributions
699 of multiple species of larval tuna off the Nansei Islands, Japan, *Mar. Ecol. Prog. Ser.*, 636, 123-
700 137, doi:10.3354/meps13216.

701 Thuiller, W., D. Georges, R. Engler, and F. Breiner (2020), biomod2: Ensemble Platform for Species
702 Distribution Modeling, edited.

703 Volkman, J. K., and E. Tanoue (2002), Chemical and biological studies of particulate organic matter in the
704 ocean, *J. Oceanogr.*, 58(2), 265-279, doi:10.1023/A:1015809708632.

705 Wada, E. (1980), Nitrogen isotope fractionation and its significance in biogeochemical processes
706 occurring in marine environments, in *Isotope Marine Chemistry*, edited by E. Goldberg, Y. Horibe
707 and K. Saruhashi, pp. 375-398, Uchida Rokakuho, Tokyo.

708 Wada, E., and A. Hattori (1990), *Nitrogen in the sea: forms, abundance, and rate processes*, CRC press,
709 Florida, USA.

710 Wada, E., K. Ohki, S. Yoshikawa, P. L. Parker, C. V. Baalen, G. I. Matsumoto, M. N. Aita, and T. Saino
711 (2012), Ecological aspects of carbon and nitrogen isotope ratios of cyanobacteria, *Plank. Benth.*
712 *Res.*, 7(3), 135-145, doi:10.3800/pbr.7.135.

713 Walsby, A. E. (1992), The gas vesicles and buoyancy of *Trichodesmium*, in *Marine Pelagic*
714 *Cyanobacteria: Trichodesmium and other Diazotrophs*, edited by E. J. Carpenter, D. G. Capone
715 and J. G. Rueter, pp. 141-161, Springer Netherlands, Dordrecht, doi:10.1007/978-94-015-7977-
716 3_9.

717 Watanabe, Y. W., H. Ishida, T. Nakano, and N. Nagai (2005), Spatiotemporal decreases of nutrients and
718 chlorophyll-*a* in the surface mixed layer of the western North Pacific from 1971 to 2000, *J.*
719 *Oceanogr.*, *61*(6), 1011-1016, doi:10.1007/s10872-006-0017-y.

720 Wu, J., Y. Nishijima, and H. Satake (2008), Seasonal variation of $\delta^{15}\text{N}$ of nitrate ion found in
721 precipitation at Toyama, *Chikyukagaku*, *42*(1), 1-11, doi:10.14934/chikyukagaku.42.1.

722 Wu, Y., J. Zhang, D. J. Li, H. Wei, and R. X. Lu (2003), Isotope variability of particulate organic matter at
723 the PN section in the East China Sea, *Biogeochemistry*, *65*(1), 31-49,
724 doi:10.1023/a:1026044324643.

725 Yoshikawa, C., A. Makabe, Y. Matsui, T. Nunoura, and N. Ohkouchi (2018), Nitrate isotope distribution
726 in the Subarctic and Subtropical North Pacific, *Geochemistry, Geophysics, Geosystems*, *19*(7),
727 2212-2224, doi:10.1029/2018gc007528.

728

729

Figure Captions

Figure 1. (a) Map of the sampling site and depiction of the flow of the Kuroshio current. Light and dark gray lines represent the normal path (January 2015) and large-meandered path of the Kuroshio current (January 2018), respectively. Black line depicts the “O-line”. (b) Temporal variations of the sampling stations (circles and squares) and the Kuroshio axis (solid gray line) along the O-line. Light and dark gray boxes in (b) denote the summer and winter periods, respectively. Closed circles and squares correspond to the coastal and offshore stations, respectively.

Figure 2. Seasonal variations in (a) temperature, (b) salinity, (c) chlorophyll *a* concentration, (d) nitrate concentration, and (e) carbon-based diatom abundance. Dark blue and green boxes indicate the data in the coastal and offshore areas, respectively. Chlorophyll *a* concentration, nitrate concentration, and carbon-based diatom abundance are converted into common-logarithm values. Each boxplot shows the medians (vertical thick lines within boxes), upper and lower quartiles (boxes), quartile deviations (horizontal bars), and outliers (circles).

Figure 3. Scatter diagrams for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of POM along the O-line in the (a) coastal and (b) offshore areas. Circles and crosses indicate the samples collected in the coastal and offshore areas, and the colors denote the different seasons. The ellipses include 95% probability ellipse for each season and area.

Figure 4. Relationship between $\delta^{13}\text{C}$ 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).

Diatom abundance data is available at only 10-m depth. The colors and shapes denote the areas and seasons, respectively (blue: coastal; green: offshore; closed circle: winter; open triangle: spring; cross: summer; closed square: autumn). The gray solid dashed line with shadow represents the linear regression line and 95% confidence values. When the linear regression line is the dashed line, the linear regression relationship is not significant ($p > 0.05$).

Figure 5. Relationship between $\delta^{15}\text{N}$ 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). Diatom abundance data is available at only 10-m depth. The colors and shapes denote the areas and seasons, respectively (blue: coastal; green: offshore; closed circle: winter; open triangle: spring; cross: summer; closed square: autumn). The gray solid dashed line with shadow represents the linear regression line and 95% confidence values. When the linear regression line is the dashed line, the linear regression relationship is not significant ($p > 0.05$).

Figure 6. Responses of the least-AIC GLMs to $\delta^{13}\text{C}$ in terms of (a) temperature, (b) salinity, (c) log-chlorophyll, (d) log-nitrate, (e) latitude of the Kuroshio axis, and (f) log-diatom. Solid and dotted lines denote the GLMs of $\delta^{13}\text{C}$ fitting with the linear and quartic functions, respectively. Blue and green vertical ticks on the x-axes indicate the data points of the coastal and offshore areas, respectively. The numerical values of common-log transformed values (c, d, and f) are re-transformed to the normal values, but the axes are shown with the common-log transformed values.

Figure 7. Responses of the least-AIC GLMs to $\delta^{15}\text{N}$ in terms of (a) temperature, (b) salinity, (c) log-chlorophyll, (d) log-nitrate, (e) latitude of the Kuroshio axis, and (f) log-diatom. Solid and dotted lines denote the GLMs of $\delta^{15}\text{N}$ fitting with the linear and quartic functions, respectively. Blue and green vertical ticks on the x-axes indicate the data points of the coastal and offshore areas, respectively. The numerical values of common-log transformed values (c, d, and f) are re-transformed to the normal values, but the axes are shown with common-log transformed values. Gray horizontal lines denote that the parameter is not selected in the least-AIC GLMs.

Figure 8. Yearly variations in the least square means (lsmean, closed circle) of $\delta^{13}\text{C}$ (a, b) and $\delta^{15}\text{N}$ (c, d) based on the linear GLM (a, c) and quartic GLM (b, d). Vertical lines denote the $2 \times$ standard errors (*i.e.*, 95% confidence intervals).

Figure 9. Scatter plot of the calculated climatological $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in each season and area based on the seasonal and spatial median values. The colors denote the seasons, and the shapes denote the combination of areas (open: offshore; closed: coastal) and models (square: linear function GLMs; circle: quartic function GLMs).

Figure 1.

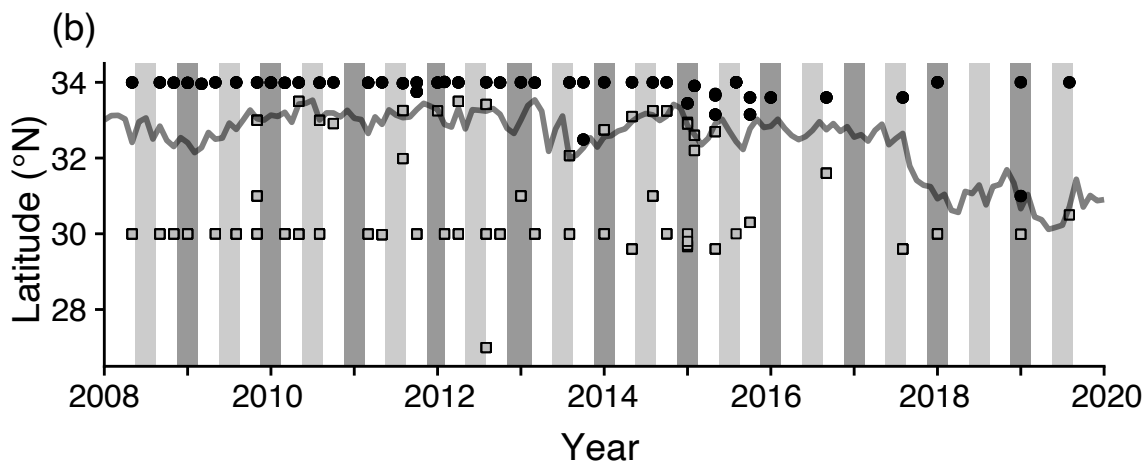
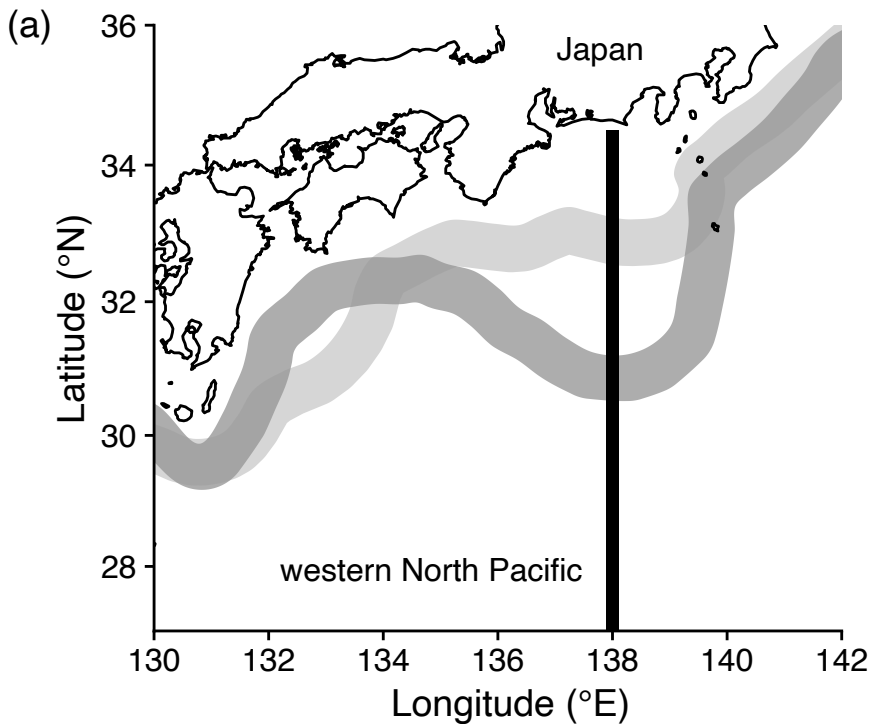


Figure 2.

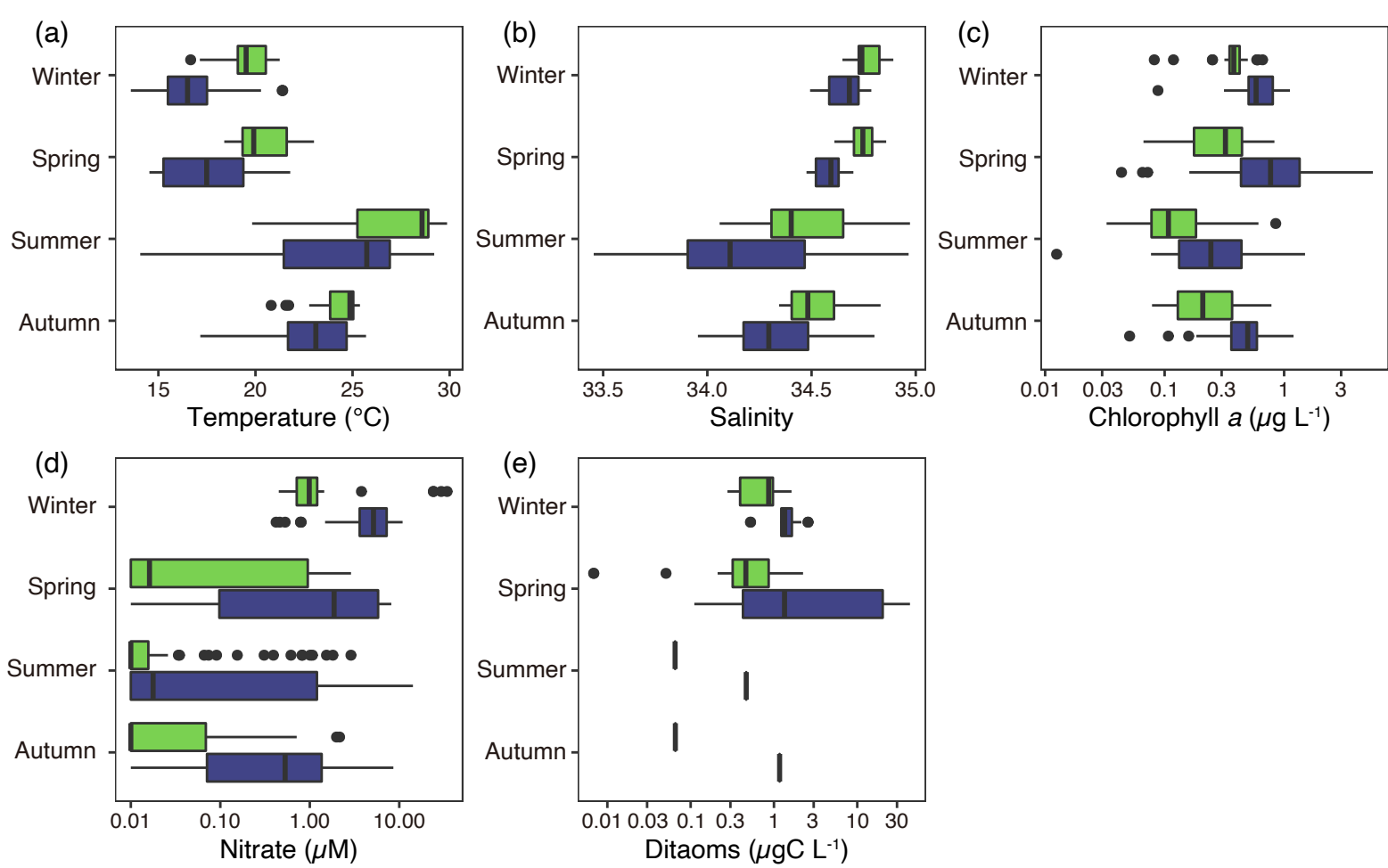


Figure 3.

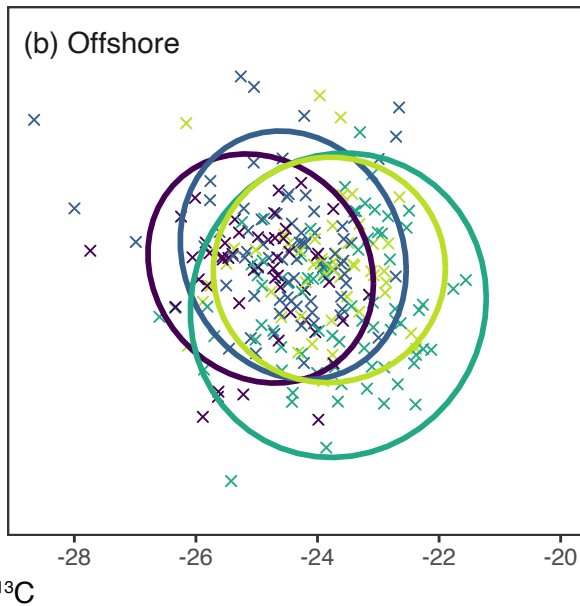
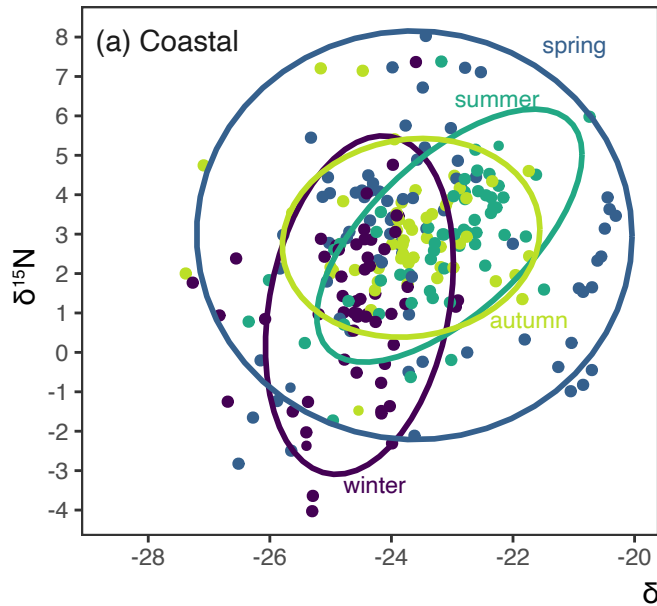


Figure 4.

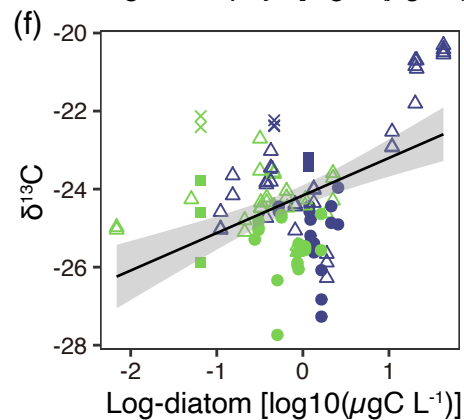
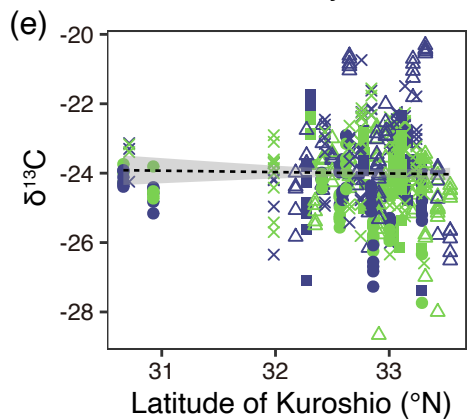
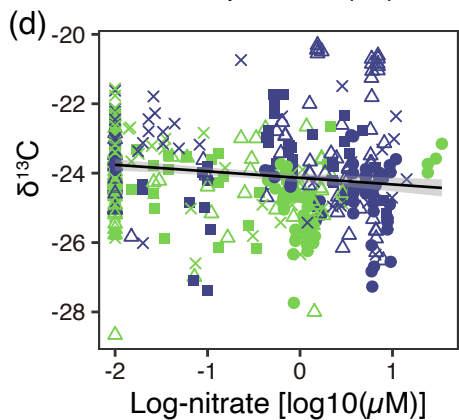
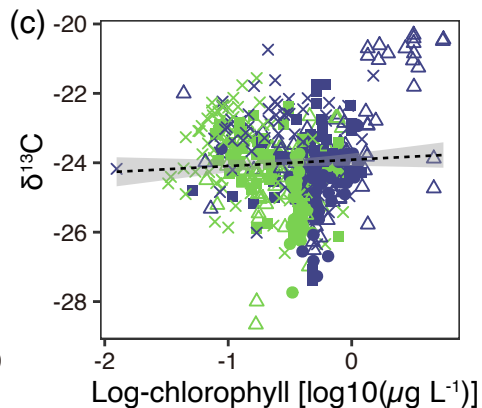
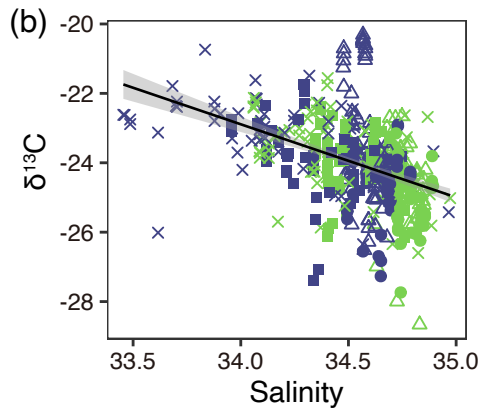
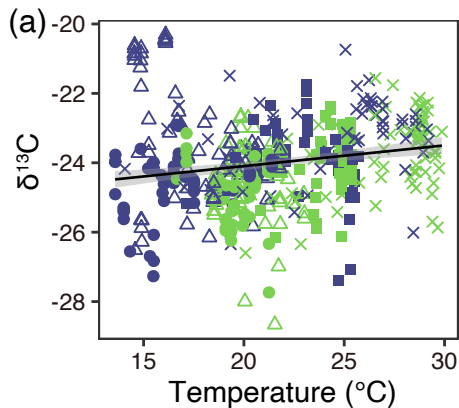


Figure 5.

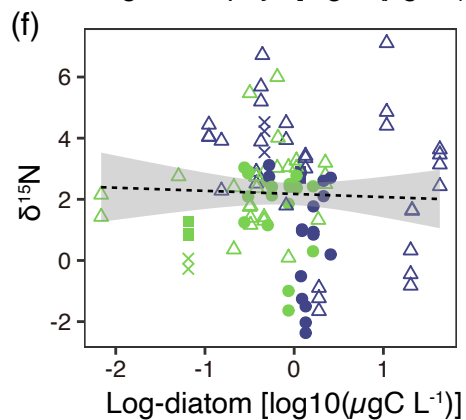
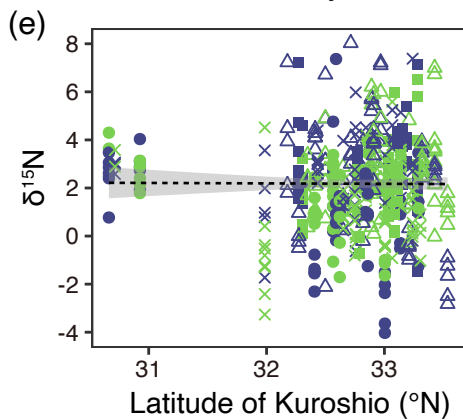
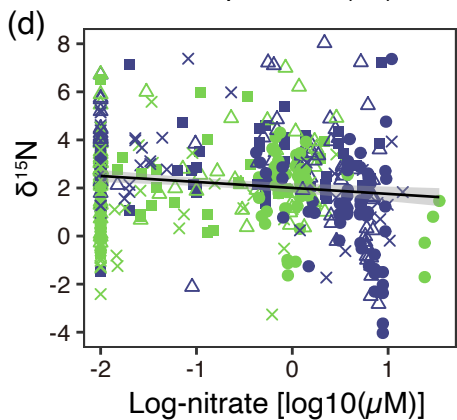
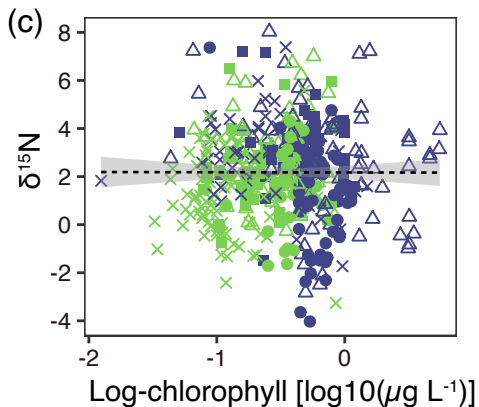
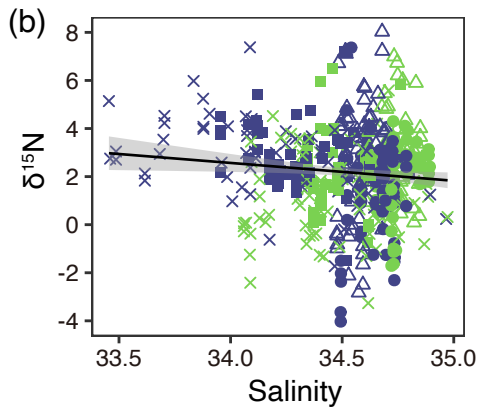
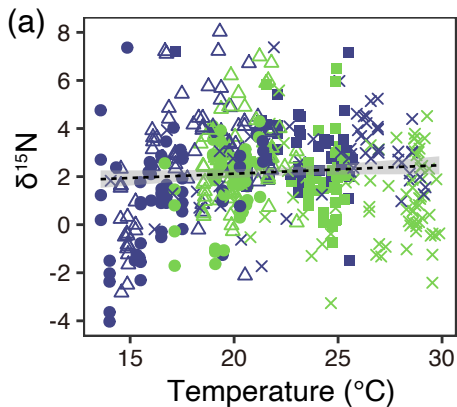


Figure 6.

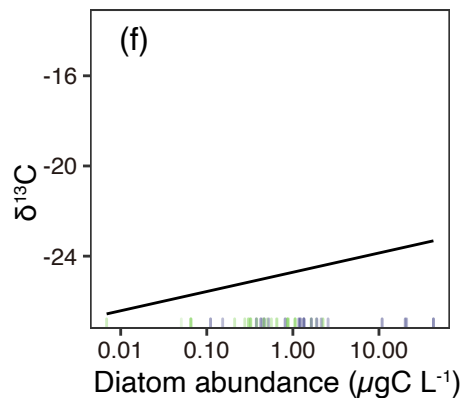
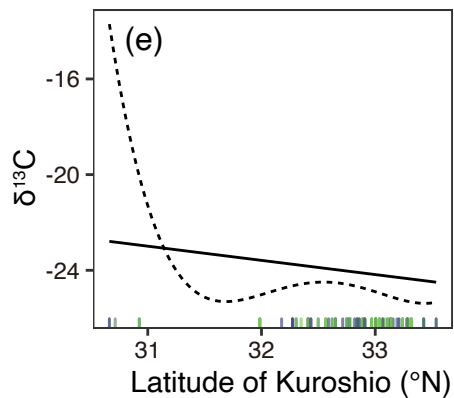
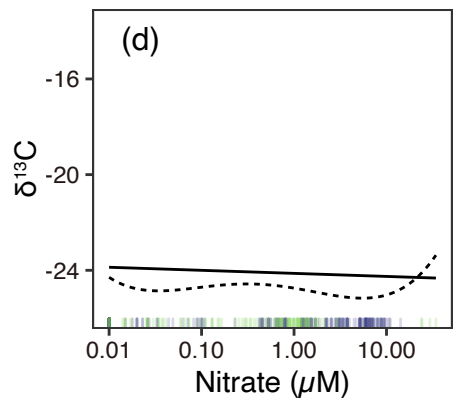
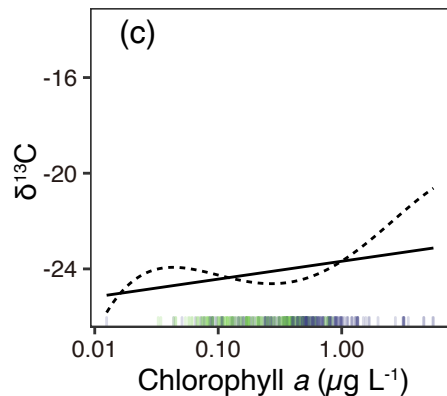
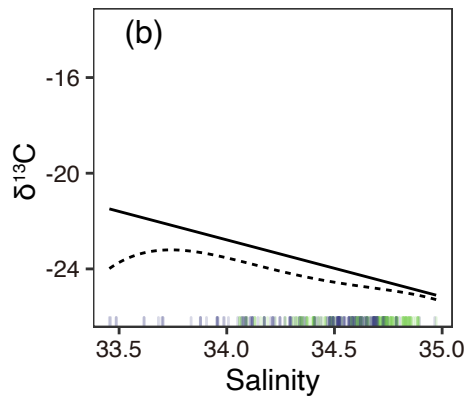
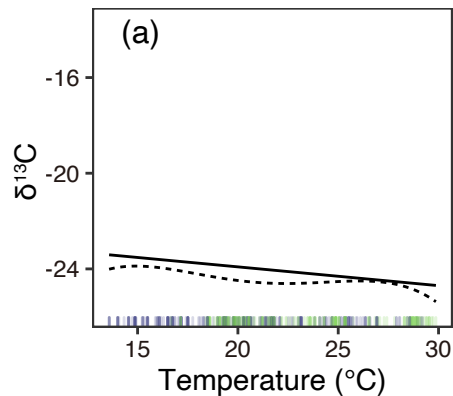


Figure 7.

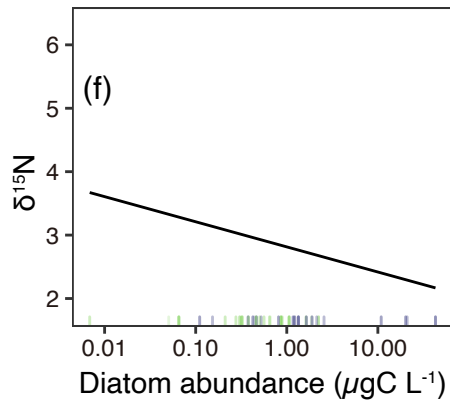
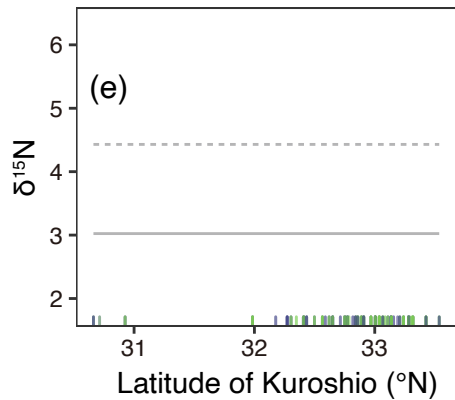
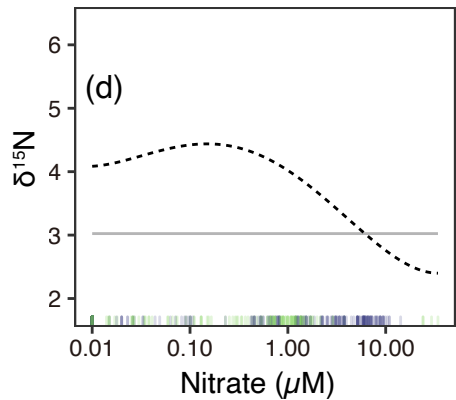
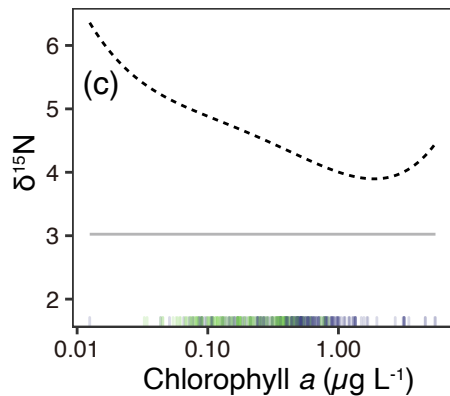
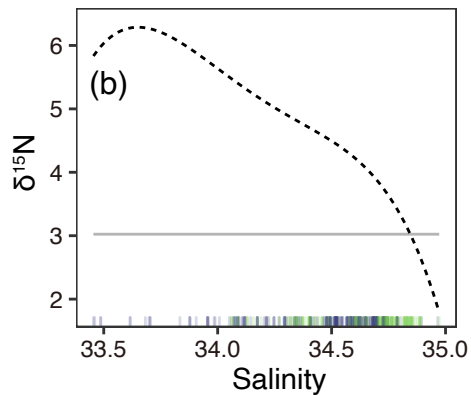
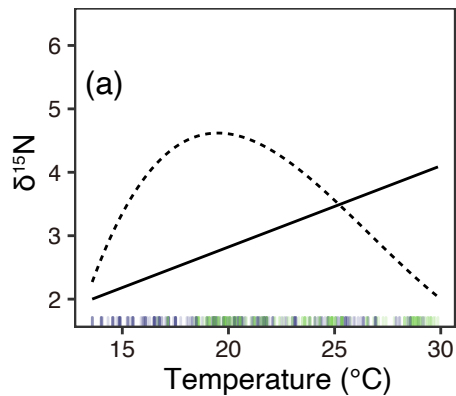


Figure 8.

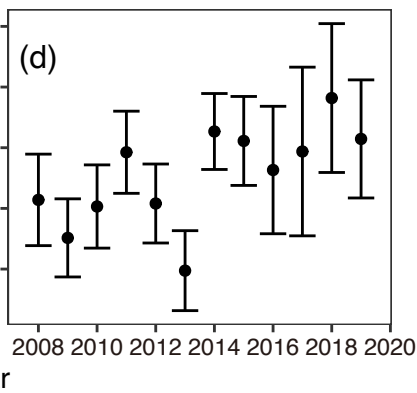
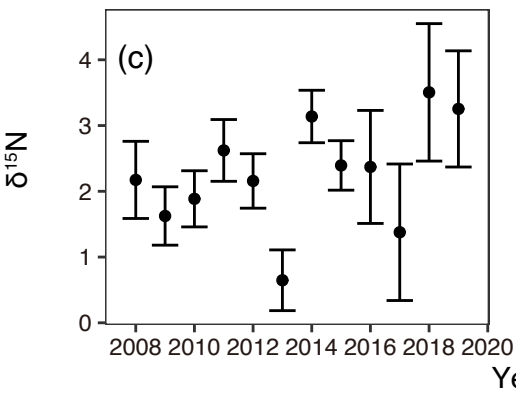
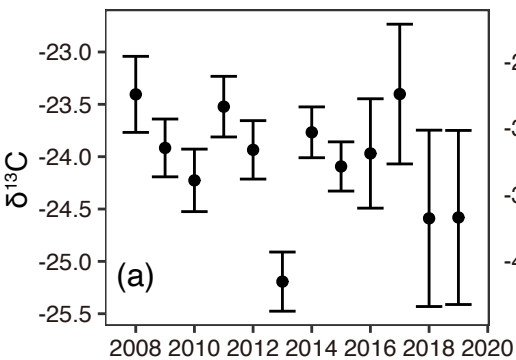


Figure 9.

