



Supplement of

Carbon and nitrogen dynamics in the coastal Sea of Japan inferred from 15 years of measurements of stable isotope ratios of *Calanus sinicus*

Ken-ichi Nakamura et al.

Correspondence to: Taketoshi Kodama (blc_kodama@hotmail.com)

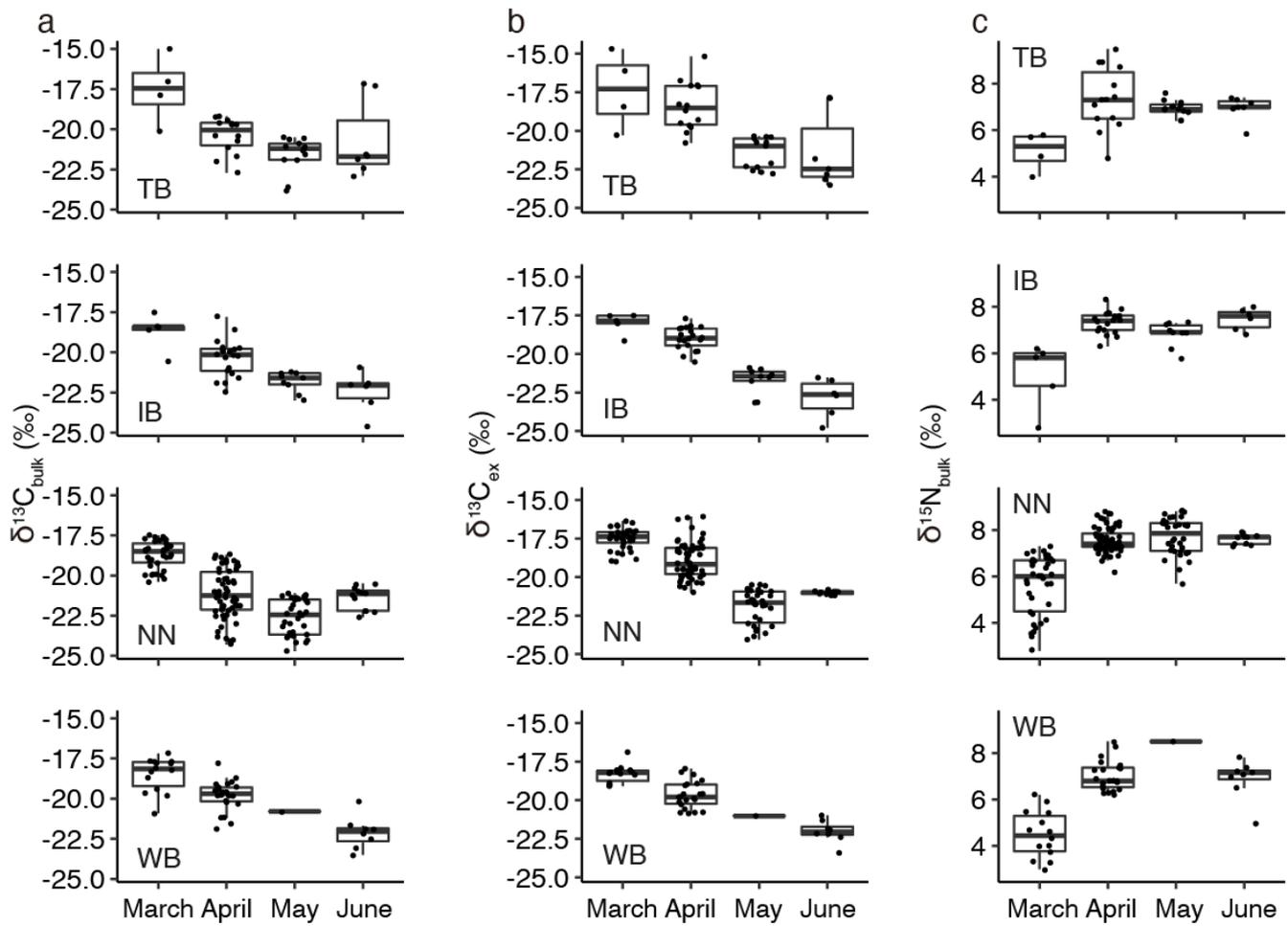
The copyright of individual parts of the supplement might differ from the article licence.

Materials and methods

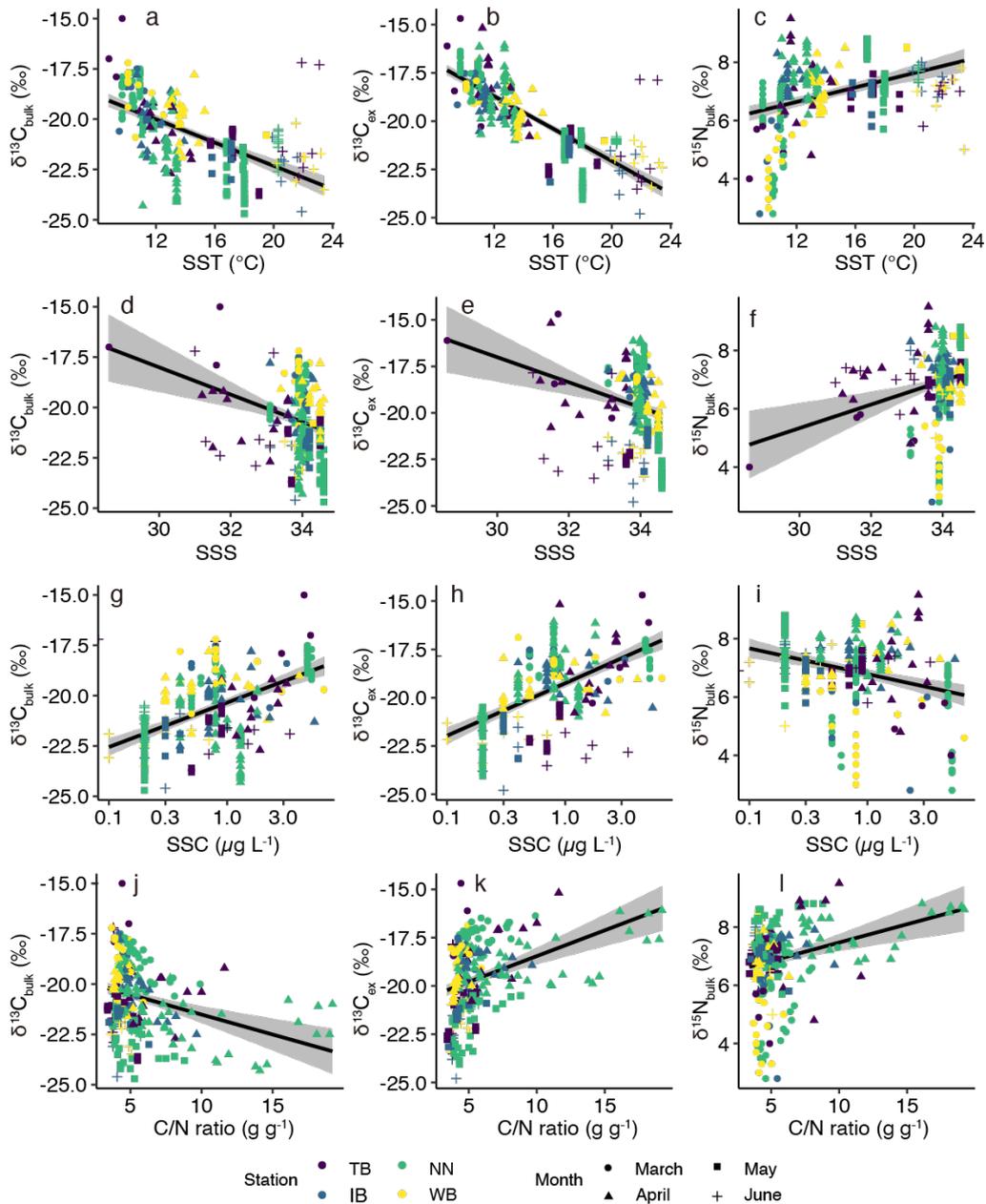
In this supplement, the results which applied simple statistical analyses for identification of the spatial, monthly and stage [copepodite stage V (C5), adult female (F) and adult male (M)] difference of $\delta^{13}\text{C}_{\text{bulk}}$, $\delta^{13}\text{C}_{\text{ex}}$ and $\delta^{15}\text{N}_{\text{bulk}}$, and the relationships between the $\delta^{13}\text{C}_{\text{bulk}}$, $\delta^{13}\text{C}_{\text{ex}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ of *Calanus sinicus* and environmental parameters [sea surface temperature (SST), sea surface chlorophyll *a* concentration (SSC), sea surface salinity (SSS) and ratio of carbon: nitrogen (C/N ratio)]. We applied ANOVA (analysis of variance) with Tukey's test for identifying the spatial and monthly difference. At some stations, we measured the $\delta^{13}\text{C}_{\text{bulk}}$ or $\delta^{15}\text{N}_{\text{bulk}}$ of multiple subsamples, and we did the statistical analyses without averaging data from the same station.

Results

10 Significant monthly variations were observed for both $\delta^{13}\text{C}_{\text{bulk}}$, lipid-free $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{ex}}$) and $\delta^{15}\text{N}_{\text{bulk}}$ at every station (ANOVA, $p < 0.001$, Fig. S1). The highest $\delta^{13}\text{C}_{\text{bulk}}$ values were observed in March at all four stations (TB: $-17.5 \pm 2.1\%$; IB: $-18.7 \pm 1.1\%$; NN: $-18.7 \pm 0.9\%$; and WB: $-18.5 \pm 1.1\%$, Fig S1a). The monthly mean $\delta^{13}\text{C}_{\text{bulk}}$ values decreased from March to May at all four stations, although there was only one subsample at WB in May (Fig. S1a). In June, monthly mean $\delta^{13}\text{C}_{\text{bulk}}$ values were similar to those in May, except at NN. The mean $\delta^{13}\text{C}_{\text{bulk}}$ at NN was significantly lower in May ($-22.4 \pm 2.2\%$) than in June ($-21.2 \pm 1.3\%$) (ANOVA with Tukey's HSD test, $p = 0.028$). The monthly mean values were significantly different among stations in April and March (ANOVA, $p < 0.006$). In all four months, the $\delta^{13}\text{C}_{\text{bulk}}$ at NN was the lowest among the four stations, and there were significant differences between the $\delta^{13}\text{C}_{\text{bulk}}$ values at WB and at NN in April and between those at TB and NN in May (ANOVA with Tukey's HSD test, $p < 0.006$). The monthly and spatial variations of $\delta^{13}\text{C}_{\text{ex}}$ in *C. sinicus* were similar to those of $\delta^{13}\text{C}_{\text{bulk}}$. The highest $\delta^{13}\text{C}_{\text{ex}}$ values were observed in March at all four stations, which was the same as $\delta^{13}\text{C}_{\text{bulk}}$ (Fig. S1b). The monthly mean $\delta^{13}\text{C}_{\text{ex}}$ values decreased from March to May or June at all four stations (Fig. S1b). The lowest monthly mean $\delta^{15}\text{N}_{\text{bulk}}$ was observed in March at all four stations (TB: $5.1 \pm 0.8\%$; IB: $5.1 \pm 1.4\%$; NN: $5.6 \pm 1.3\%$; and WB: $4.5 \pm 1.0\%$). The $\delta^{15}\text{N}_{\text{bulk}}$ values were significantly elevated in April (TB: $7.4 \pm 1.3\%$; IB: $7.3 \pm 0.5\%$; NN: $7.5 \pm 0.5\%$; and WB: $7.0 \pm 0.7\%$), and they were stable from April to June (ANOVA with Tukey's HSD test, $p > 0.2$) at all four stations (Fig. S1c). The differences of the monthly values among stations were significant during all four months (ANOVA, $p < 0.037$): monthly $\delta^{15}\text{N}_{\text{bulk}}$ values at NN were highest among the four stations in every month. In March, April, and June, the $\delta^{15}\text{N}_{\text{bulk}}$ values were significantly lower at WB than at NN (ANOVA with Tukey's HSD test, $p < 0.03$), but there were no significant differences between other pairs of stations. In May, the values were significantly lower at TB and IB than at NN (ANOVA with Tukey's HSD test, $p < 0.003$). There were significant linear relationships between the $\delta^{13}\text{C}_{\text{bulk}}$, $\delta^{13}\text{C}_{\text{ex}}$, and $\delta^{15}\text{N}_{\text{bulk}}$ values and SST, logarithm-transformed SSC, SSS and C:N ratio. The nature of the relationships was similar between $\delta^{13}\text{C}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{ex}}$, but those differed between $\delta^{13}\text{C}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ (Fig. S2). The Pearson's correlation coefficient was negative between $\delta^{13}\text{C}_{\text{bulk}}$ or $\delta^{13}\text{C}_{\text{ex}}$ and SST ($p < 0.001$, Fig S2a, b), negative between $\delta^{13}\text{C}_{\text{bulk}}$ or $\delta^{13}\text{C}_{\text{ex}}$ and SSS ($p < 0.001$, Fig S2d, e) and positive between $\delta^{13}\text{C}_{\text{bulk}}$ or $\delta^{13}\text{C}_{\text{ex}}$ and logarithm-transformed SSC ($p < 0.001$, Fig S2g, h). In contrast, Pearson's correlation coefficients were positive between $\delta^{15}\text{N}_{\text{bulk}}$ and both SST ($p < 0.001$, Fig S2c) and SSS ($p < 0.001$, Fig S2f), and it was negative between $\delta^{15}\text{N}_{\text{bulk}}$ and logarithm-transformed SSC ($p < 0.001$, Fig 5i). When an outlier value of SSS was removed (SSS was 28 in March at TB), the correlations with SSS were still significant. The C/N ratios of *C. sinicus* were negatively and positively correlated with $\delta^{13}\text{C}_{\text{bulk}}$ and $^{13}\text{C}_{\text{ex}}$, respectively (both $p < 0.001$, Fig. S2j, k). The C/N ratio was positively correlated with $\delta^{15}\text{N}_{\text{bulk}}$ ($p < 0.01$, Fig S2l).



40 **Figure S1: Variations of (a) $\delta^{13}\text{C}_{\text{bulk}}$, (b) $\delta^{13}\text{C}_{\text{ex}}$, and (c) $\delta^{15}\text{N}_{\text{bulk}}$ values of *C. sinicus* among months and stations. The variations at TB, IB, NN, and WB are shown. Boxplots show the means (horizontal lines within boxes), standard deviations (boxes), and maximum or minimum values (bars). Small points are raw data points.**



45 **Figure S2: Relationships between (a) $\delta^{13}\text{C}_{\text{bulk}}$ and sea surface temperature (SST), (b) $\delta^{13}\text{C}_{\text{ex}}$ and SST, (c) $\delta^{15}\text{N}_{\text{bulk}}$ and SST, (d) $\delta^{13}\text{C}_{\text{bulk}}$ and sea surface salinity (SSS), (e) $\delta^{13}\text{C}_{\text{ex}}$ and SSS, (f) $\delta^{15}\text{N}_{\text{bulk}}$ and SSS, (g) $\delta^{13}\text{C}_{\text{bulk}}$ and sea surface chlorophyll *a* concentration (SSC), (h) $\delta^{13}\text{C}_{\text{ex}}$ and SSC, (i) $\delta^{15}\text{N}_{\text{bulk}}$ and SSC, (j) $\delta^{13}\text{C}_{\text{bulk}}$ and carbon: nitrogen ratio (C/N ratio), (k) $\delta^{13}\text{C}_{\text{ex}}$ and C/N ratio, and (l) $\delta^{15}\text{N}_{\text{bulk}}$ and C/N ratio.** The colors and shapes of points indicate stations and sampling months, respectively. The black lines and gray shading indicate linear regression lines and 95% confidence intervals, respectively. The SSC values (g–h) were transformed as logarithm values.

50