Linear relationship between carbon and nitrogen isotope ratios along simple food chains in marine environments

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ABSTRACT

To examine the relationship between carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of zooplankton, we analyzed samples collected bimonthly from March to October 2009, from the euphotic layers of the Oyashio current along the A-line in the western North Pacific. Isotopic ratios of higher trophic levels such as predatory zooplankton and/or long-lived zooplankton varied little with season, while those of short-lived zooplankton were variable on the $\delta^{15}N$ - $\delta^{13}C$ map. We also analyzed preserved samples taken from the warm-core ring 86-B which derived from the Kuroshio extension region. Although the zooplankton groups in the two regions exhibited different values in $\delta^{15}N$, the $\delta^{15}N$ versus $\delta^{13}C$ slopes for each ecosystem do not show significant difference.

Statistical analysis conducted together with previously published data from the Antarctic Ocean and the Gulf of Alaska suggested a commonness in $\delta^{15}N$ versus $\delta^{13}C$ slope throughout the four regions. We attributed this common slope to physiological aspects of feeding processes (*e.g.,* the kinetic isotope effects inherent in the processes of amino acid synthesis). The common pattern for all four oceanic regions suggests that stable isotopes may be used to elucidate general patterns in ecosystems and biogeochemical cycles.

Key words: stable isotopes, nitrogen, carbon, food chain, isotopic fractionation

1. INTRODUCTION

Over the past 20 years, rapid progress in satellite remote sensing, automatic field observation systems, computer simulation, and the development of ecosystem analytical methods such as stable isotope (SI) studies and genomic sequencing have allowed ecologists to observe natural ecosystems from entirely new perspectives. For example, using satellite-derived data, we can clarify the distributions of phytoplankton community structures and primary production on the global scale (Behrenfeld and Falkowski, 1997; Behrenfeld *et al.*, 2005; Alvain *et al.*, 2008; Hirata and Brewin, 2009; Okamoto *et al.*, 2010). Considerable progress has also been made in marine ecosystem modeling, although there remain large discrepancies in ecosystem structure between models and observations (Aita *et al.*, 2007; Sumata et al., 2010). For ecosystem structure, complex processes within food chains are elucidated by energy flux, but this does not provide information at species, metabolic, or molecular levels. Genetic analysis is mainly applied to clarify positions on phylogeny and ecosystem evolution. On the other hand, SI analysis is a useful tool for the study of biogeochemical cycles as well as ecosystem structures. We can determine the structure of food webs and the interactions between organisms using

distributions and variation in C/N isotope ratios together with their fractionations (Wada, 2009).

Variation in the SI ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are well studied for single-feeding processes: $\delta^{15}N$ is enriched by about 3‰ to 4‰ per trophic level (TL) (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Fry, 1988). In subtropical and tropical seas, where phytoplankton grow under nitrogen-deficient conditions in the euphotic layer, blue-green algae fix molecular nitrogen and exhibit $\delta^{15}N$ values of approximately -2‰ (Minagawa and Wada, 1986; Carpenter *et al.*, 1997). At high latitudes, phytoplankton $\delta^{15}N$ exhibit rather low values of -1‰ to 3‰ by nitrogen isotope fractionation under high nitrate concentrations with $\delta^{15}N$ of ca. 6‰, whereas nitrate $\delta^{15}N$ increases with decreasing utilization of nitrate by phytoplankton (e.g. Sigman and Casciotti, 2001). The $\delta^{15}N$ values of algae differ widely from -1‰ to 10‰ depending on the forms of inorganic nitrogen utilized. Wada and Hattori (1991) broadly divided phytoplankton in the North Pacific into three types based on $\delta^{15}N$ and the three major forms of nitrogenous compounds utilized: NO_3^-, NH_4^+, and N_2. In marine environments, the primary producer (phytoplankton) provides the base of the food chain for zooplankton and fishes, in turn affecting the $\delta^{15}N$ values of animals at higher trophic levels. Chiba *et al.* (2009) examined regional differences in $\delta^{15}N$ of major copepod species in the subarctic North Pacific. Areal $\delta^{15}N$ of *Neocalanus spp.* was high in the western and eastern North Pacific (7‰–10‰) and low in the central North Pacific (2‰–4‰) reflecting the gradient of nitrate concentration.

Phytoplankton have lower $\delta^{13}C$ values than coastal C4 plants, such as eelgrass (average $\delta^{13}C$ is about -10‰, generally ranging from -15‰ to -3‰; Fry and Sherr, 1984). This difference is used to determine the carbon source of the primary producer and consumer

and whether the carbon source is terrestrial, coastal, or open-ocean (Fry, 2006). For $\delta^{13}C$ in the ocean, McConnaughey and McRoy (1979) investigated food-web structure from the viewpoint of fractionation of carbon isotopes in the Bering Sea. They reported that stepwise $\delta^{13}C$ enrichment is 1.5‰ per TL. Rau *et al*. (1983) showed that the average increase in $\delta^{13}C$ per TL ranged from 0.7‰ to 1.4‰ for samples from eastern equatorial Pacific and California coastal waters, whereas about 1‰ $\delta^{13}C$ per TL was reported for rearing systems and sea grass meadows (DeNiro and Epstein, 1978; Rau *et al.*, 1983; Fry and Sherr, 1984).

Evidence suggests that SIs have the potential to reveal complex interactions, including trophic interactions and energy or mass flow through ecological communities (Peterson and Fry, 1987; Kling *et al*., 1992; Cabana and Rasmussen, 1996). However, most precise examinations of trophic fractionation have been mostly limited to freshwater ecosystems (Vander Zanden and Rasmussen, 1999; Post *et al*., 2000; Post, 2002). At present, the magnitude of trophic fractionation of carbon isotopes in natural ecosystems remains unclear and requires further study with emphasis on kinetic isotope fractionation during feeding processes in marine ecosystems. For example, $\Delta\delta^{13}C$ per TL seems to be low in lakes and coastal areas and higher in pelagic areas of the ocean (Post, 2002; Wada *et al.*, 1987).

The $\delta^{15}N$ and $\delta^{13}C$ of animals are thus greatly affected by their feeding processes within the food chain. Based on this fact, we focus on the ratio of C and N trophic fractionation per TL ($\Delta\delta^{15}N/\Delta\delta^{13}C$), which could be obtained as the slope between different TLs in plots of $\delta^{15}N$ versus $\delta^{13}C$. In this study, we analyzed isotopic ratios of plankton samples taken from the Oyashio waters and from Warm-core Ring 86-B. The results of isotopic ratios and comparison with $\delta^{15}N$ and $\delta^{13}C$ ratios for both regions are

described in the 'Results' section. In the 'Discussion' section, we discuss the $\Delta\delta^{15}N/\Delta\delta^{13}C$ slope using our data combined with data from the Antarctic Ocean (Wada et al., 1987) and the Gulf of Alaska (Kaeriyama, 2004). If we could better understand both carbon and nitrogen trophic fractionation within ecosystems, SIs may help to elucidate general patterns in ecosystems and biogeochemical cycles.

2. MATERIALS AND METHODS

Study Area (Oyashio)

Plankton samples were collected in the Oyashio waters along the A-line monitoring transect (38º00'N to 42º50'N, 144º50'E to 147º50'E) of the Fisheries Research Agency (FRA) of Japan. The transect extends from the cold Oyashio current to the warm Kuroshio current in the western North Pacific (Fig. 1-(a); Saito *et al.*, 2002). The Oyashio flows southward along the Kuril Islands, and its first and second branches fork near Sanriku, Japan. In this region, approximately 100-km-long warm eddies, called warm-core rings (WCR), extend from the Kuroshio current (Okuda, 1991).

Samples were collected along the A-line (Fig. 1) bimonthly from March to October 2009, during cruises of the R.V. *Wakataka-maru* of the Tohoku National Fisheries Research Institute (FRA, WK-09-03 and WK-09-07 cruises), the R.V. *Kaiyo-maru* of the Fisheries Agency (KY-09-07 cruise), and the R.V. *Hokko-maru* of the Hokkaido National Fisheries Research Institute, FRA (HK-09-10 cruise).

Study Area (Warm-core Ring 86-B)

Samples from the WCR, which formed from the meanders of the Kuroshio extension in mid-March 1986 (Fig.1-(b); Saino, 1992; Tsuda and Nemoto, 1992), were collected during the WCR 86-B cruise KH-87-4 of the *Hakuho-Maru* of the University of Tokyo,

from 1 to 25 September 1987. In the WCR, thermostad waters of about 10ºC were observed from 75 to 350 m at the center of the water mass (Saino, 1992), and water column nitrate concentrations were less than 0.2 μM. Samples were collected from the surface and 600-m depth using 10 horizontal Motoda (MTD) nets (Motoda, 1971) of 56-cm mouth diameter and 0.33-mm mesh size (Tsuda and Nemoto, 1992; Hasumoto, 2006). After collection, all samples were desiccated and preserved in sealed glass containers. We used zooplankton samples from the upper 150 m for analysis of isotopic ratios and comparison with $\delta^{15}N$ and $\delta^{13}C$ ratios of the Oyashio.

Temperature and nitrate and chlorophyll* a *concentrations (Oyashio)

Vertical temperature profiles were measured at all stations with CTD (conductivity-temperature-depth; Sea Bird Electronics, Washington, USA), and water samples for analyses of nitrate and chlorophyll *a* concentrations were collected using Niskin bottles. Water samples for nitrate were immediately frozen at -20ºC until analysis using a nutrient analyzer AACS-III (BRAN+LUEBBE, Norderstedt, Germany). Water samples were filtered with Whatman GF/F filters for chlorophyll *a* analysis; collected samples underwent extraction with *N*, *N*-dimethylformamide (DMF). Fluorescence was then measured using a fluorometer (Turner Designs, Sunnyvale, California, USA; Holm-Hansen *et al.*, 1965; Kasai *et al.*, 2001; Saito *et al.*, 2002). Along the A-line, we identified the Oyashio waters based on temperatures at 100-m depth, following Kawai (1972) and Odate (1994); water masses with temperatures below 5ºC at 100-m depth were classified as the Oyashio waters.

Dominant zooplankton taxa (Oyashio)

In the Oyashio region, copepods are most the important species, constituting over 80%

of total mesozooplankton biomass, followed by chaetognaths, euphausiids, and amphipods (Ikeda *et al*., 2008). Four species of chaetognaths (*Sagitta elegans*, *Eukrohnia hamata*, *E. bathypelagica*, and *E. fowler*) occur in the Oyashio waters, and only *S. elegans* occurs shallower than 250 m (e.g. Ikeda *et al*., 2008). Kim *et al*. (2009) found that 80% of the abundance of three species of euphausiids in the Oyashio region was composed of *Euphausia pacifica*. Among copepods, the four dominant species, *Neocalanus cristatus*, *N. flimingeri*, *N. plumchrus*, and *Eucalanus bungii*, exhibit different life cycles and vary seasonally in abundance (Tsuda *et al.*, 1999; Mackas and Tsuda, 1999; Kobari *et al.*, 2003). In particular, the life histories of *E. bungii* and *Neocalanus spp.* differ substantially. For *Neocalanus spp.*, the peak abundance of *N. cristatus* C5 occurred from May to July, when lipid storage was primarily observed in the C5 stage (Tsuda *et al.*, 2004). Mature populations descend to below 500-m depth and spawn in October to December in the Oyashio region. Hatched populations in the deep layer ascend to the surface layers and continue to graze and grow into adults in the mixed layer. Larger *N. cristatus* remain for approximately 6 months in the mixed layer, but smaller *N. flimingeri* stay for about 1.5 months (Miller *et al*., 1984; Kobari and Ikeda, 1999; Tsuda *et al.*, 1999, 2004). *Eucalanus bungii*, on the other hand, spawns near the surface layers during the spring bloom. Larvae of *E. bungii* prey on phytoplankton during the season of high primary production and algal blooms (Miller *et al.*, 1984; Tsuda *et al.*, 2004; Kobari *et al.*, 2007).

Collection of plankton samples (Oyashio)

We collected specimens of copepods, amphipods, euphausiids, and chaetognaths for SI analyses, with particular emphasis on dominant species in the western North Pacific.

Samples were collected from 150-m depth to the surface using vertical tows of a NORPAC net (45-cm mouth diameter, 0.33-mm mesh size) at a speed of 0.5 m s^{-1}. After collection, samples were filtered through a 0.33-mm mesh net; plankton on the net were immediately frozen at -80ºC onboard until analysis. In the laboratory, all samples were thawed and rinsed quickly with a NaCl solution as isotonic seawater to remove bicarbonate (HCO_3^-) or inorganic carbon salts and were then sorted. Copepods were classified under a stereomicroscope into four species: *N. cristatus*, *N. flimingeri*, *N. plumchrus*, and *E. bungii*. We used copepodite stage C5 for isotopic analysis. A considerable amount of algae was also collected in May by vertically towing a NORPAC net from 150 m to the surface. Algae were sorted using tweezers under a stereomicroscope and were also analyzed for isotopic ratios.

Sample Preparation (Oyashio and WCR 86-B)

Zooplankton and algal samples were dried at 60ºC for more than 12 h. All samples were ground into a fine powder using an agate mortar and pestle. Lipid fractions were removed because of their generally low $\delta^{13}C$ values relative to whole organisms (DeNiro and Epstein, 1977; Monson and Hayes, 1982). Lipids were extracted and removed from samples with 1 mL methanol, 1 mL dichloromethane/methanol (7:1), and 1 mL dichloromethane/methanol (10:1) using an ultrasonicator (20 min) prior to isotopic analyses (Ohkouchi *et al.*, 1997).

Isotopic analysis (Oyashio and WCR 86-B)

Carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) of zooplankton and algae were determined using an elemental analyzer/isotope-ratio mass spectrometer (EA/IRMS, ThermoFinnigan FlashEA1112, ConFloIII, DeltaPlusXP; Ohkouchi *et al.*, 2005; Ogawa

et al., 2010) at the Institute of Biogeosciences, Japan Agency for Marine–Earth Science and Technology (JAMSTEC), the Japan Chemical Analysis Center (Thermo Fisher Scientific, Flash2000, GC-IsoLink, Delta V advantage), or at SI Science Co., Ltd. (Thermo Fisher Scientific, Flash2000, ConFloIV, Delta V). The lipid-free samples were transferred to pre-cleaned tin capsules and then introduced into the EA/IRMS system. Isotope values are reported in standard □-notation relative to the international standards:

$$\delta X\ (‰) = (R_{sample}/R_{standard} - 1) \times 10^3 ,$$

where X is ^{13}C or ^{15}N, and R is the isotopic ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and standard, respectively. Samples were referenced to the following standards: Vienna-PeeDee Belemnite limestone (VPDB) for carbon and atmospheric nitrogen (AIR) for nitrogen. International and/or in-house standard materials (tyrosine, proline, alanine, and glycine) were measured alongside samples to calibrate the isotope data (Ogawa *et al.*, 2010; Sato and Suzuki, 2010). The analytical errors associated with the standard materials were less than ±0.2‰ for both carbon and nitrogen.

Data analysis (Oyashio and WCR 86-B)

In the Oyashio waters, seasonal differences in isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$) of zooplankton were analyzed using one-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA). ANCOVA was used to test for differences among the slopes ($\delta^{15}N/\delta^{13}C$) of the regression lines among seasons or oceanic regions. Regression analysis was used to examine the overall trend in the $\delta^{15}N$ and $\delta^{13}C$ of animals in food chains. $\delta^{13}C$ was treated as a covariate, and sampling region was the independent variable. The

interaction between $\delta^{13}C$ and sampling region had no significant effects on the $\delta^{15}N$ of the samples and was therefore discarded from the analyses. In cases where ANCOVA tests were significant, the overall significance of differences among the sampled seasons or regions was tested using Tukey's honestly significant difference (HSD) post-hoc tests at $\alpha = 0.05$. For each sample, linear regression analysis was also applied to examine the relationships of $\delta^{15}N$ with $\delta^{13}C$. All statistical analyses were conducted using JMP software (version 8.0.2 for Windows, SAS Institute, Inc., Carey, North Carolina, USA).

3. RESULTS

Seasonal changes and environmental conditions at the A-line

We collected samples during four cruises in March, May, July, and October. Seasonal changes in average temperature as well as nitrate and chlorophyll *a* concentrations of the water columns are shown with corresponding mixed layer depths (MLDs; Fig. 2). In March, the mean water temperature of the Oyashio region at stations A2 to A5 was 1.1ºC ± 0.5ºC (mean ± SD), and nitrate concentrations were 23.4 ± 3.1 μM. The mean water temperature increased from 2.4ºC ± 0.5ºC (A2 to A6) to 12.3ºC ± 1.4ºC (A2 to A4.5) in May (spring) to July (summer), whereas nitrate concentrations decreased from 18.0 ± 4.9 μM to 6.9 ± 4.4 μM. During this time, chlorophyll *a* decreased from 4.9 ± 3.1 mg m^{-3} in May to 0.64 ± 0.33 mg m^{-3} in July. The spring bloom appeared to occur in May. In October (autumn), the mean water temperature and nitrate concentration were 12.2ºC ± 2.4ºC (A3 to A5) and 5.8 ± 2.2 μM (A3 to A7), respectively. Saito *et al.* (2002) sampled the A-line from 1990 to 1998 and found that nitrate and silicate decreased after April and was lowest in August or October.

In March and May, a warm-core ring (WCR) formed south of 42.5°N, and between A5

and A7, where the temperature of the water column increased approximately 7ºC, whereas the nitrate concentration was 10 μM. Chlorophyll *a* at the central part of the WCR, A7, was 2.1 ± 0.3 mg m^{-3}, higher than that measured in the Oyashio region (0.35 ± 0.02 mg m^{-3}) at the same time. These results show that photosynthetic activity was high in the WCR. In the WCR formed around A7 to A11 in May, the mean water temperature was approximately 8ºC higher than that of the Oyashio region at the same time, whereas the nitrate concentration decreased to approximately 8 μM at around A7. Chlorophyll *a* was 0.83 ± 0.45 mg m^{-3} in May, lower than that in the Oyashio region (4.9 ± 3.1 mg m^{-3}) and Kuroshio–Oyashio region south of A12 (2.1 ± 1.4 mg m^{-3}).

Nitrogen and carbon isotope ratios in the Oyashio waters

The $\delta^{13}C$ and $\delta^{15}N$ values for each species and sampling location in the Oyashio waters are presented in Tables I (mean value of plankton samples) and IV (list of each species and location, Appendix). The distributions of nitrogen and carbon isotope ratios of the samples of algae and dominant zooplankton species are presented in Fig. 3a and 3b. Results of isotopic data from station A1 are excluded from Table I and Fig. 3 because this station was shallow (about 100 m) and close to the Kushiro coast, Hokkaido, where it was likely affected by coastal water. Indeed, observed temperature and nitrate concentrations at A1 differed from data at other stations (Fig. 2). Euphausiids, amphipods, and chaetognaths were collected in the Oyashio region throughout the year, but copepods (C5) appeared in different seasons, depending on the species. *Neocalanus cristatus* (C5) was collected from March to July, and *N. flimingeri* (C5) and *E. bungii* (C5) were only collected in May. *Neocalanus plumchrus* (C5) was collected in May and July; it appeared at most stations in July. The $\delta^{15}N$ of amphipods differed significantly by season (ANOVA,

$F = 4.08$, $df = 3/11$, $p < 0.0356$), but that of other zooplankton did not ($p > 0.05$). On the other hand, the $\delta^{13}C$ of *N. cristatus,* euphausiids, and amphipods differed significantly among seasons (ANOVA, $F = 8.83$, $df = 2/15$, $p < 0.0029$ for *N. cristatus*; $F = 10.52$, $df = 3/13$, $p < 0.0009$ for euphausiids; $F = 8.30$, $df = 3/11$, $p < 0.0037$ for amphipods), but that of other zooplankton did not ($p > 0.05$).

In May, we collected *E. bungii* and three species of *Neocalanus spp.* (indicated by Δ in Fig. 3a). The $\delta^{15}N$ value of *E. bungii* was lowest, at 5.1 ± 0.3‰ (mean ± SD). The $\delta^{15}N$ increased in the following order: *N. cristatus*, 6.5 ± 0.5‰; *N. plumchrus*, 7.4‰; and *N. flimingeri*, 8.9 ± 0.8‰. For algae and zooplankton, the observed yearly range of $\delta^{13}C$ was -23‰ to -19‰, which was narrower than that of $\delta^{15}N$ (2‰ to 12‰). Annual mean $\delta^{15}N$ and $\delta^{13}C$ increased from copepods to euphausiids to chaetognaths (Table I). Figure 4 (a-d) presents the relationship between $\delta^{13}C$ and $\delta^{15}N$ and the linear regression line for zooplankton in the Oyashio waters. ANCOVA tests for seasonal differences of $\delta^{15}N/\delta^{13}C$ ratios among seasons (using the data in Table I) were inconclusive.

Differences in $\delta^{15}N/\delta^{13}C$ ratios of zooplankton between Oyashio and WCR 86-B

The $\delta^{13}C$ and $\delta^{15}N$ values for each species and sampling location in the WCR 86-B are presented in Tables II (mean value of plankton samples) and V (list of each species and location, Appendix). The preserved samples included salps, copepods, amphipods, and chaetognaths. Euphausiids were not observed in the upper 150 m of the WCR 86-B. For $\delta^{15}N$, amphipods had the highest value at 9.8‰, whereas copepods and chaetognaths exhibited nearly the same value (8.4 - 8.6‰). A comparison of $\delta^{15}N$ - $\delta^{13}C$ on the WCR 86-B and Oyashio maps (Fig. 5) reveals that values for zooplankton in the Oyashio varied more widely, especially for copepods. The slope of the fitted linear regression was steeper

for zooplankton from the Oyashio. However, the ANCOVA did not reveal significant differences ($p > 0.05$) between the Oyashio and WCR 86-B or conclusive evidence that their respective slopes were the same ($p > 0.05$).

4. DISCUSSION

Distributions of nitrogen and carbon isotope ratios of algae and dominant species of zooplankton

Discrepancies exist in the observed $\delta^{15}N$ and $\delta^{13}C$ values of each group of zooplankton within the Oyashio region (Fig. 3 a-b). Two factors may contribute to this result: differences in life cycle according to body size and differences in the inhabited depths. Euphausiids, which have a long life cycle of 17 to 28 months, and chaetognaths, a top predator among zooplankton, exhibited nearly the same isotopic ratios across seasons (Figs. 3 and 4). In contrast, the isotopic ratios of amphipods were similar to those of chaetognaths in March but were nearly the same as those of copepods from May to July. Zooplankton with short life spans (1 month to less than 1 year), such as copepods and amphipods, were widely spaced on the $\delta^{15}N$–$\delta^{13}C$ map (Fig. 4). Populations with large body sizes and that live in surface layers for long periods of time show less variability in their isotope ratios than populations with small body sizes that prey over shorter time spans. One reason for this pattern may be that for large-bodied populations with longer life spans, isotopic ratios reflect the mean integrated value of feeding over a longer period (Chikaraishi *et al.*, 2007); thus, the $\delta^{15}N$ of euphausiids and chaetognaths varied little with season.

The nitrate concentration in seawater changes remarkably in the mixed layer depending on changes in physical conditions and uptake of nitrate by phytoplankton.

Hence, in the present study, $\delta^{15}NO_3^-$ may increase with decreases in nitrate concentration, particularly in the euphotic layer (Saino and Hattori, 1980; Saino, 1992; Sigman and Casciotti, 2001). Isotope fractionation is correlated with growth rate (μ; Wada and Hattori, 1991; Takahashi *et al.*, 1991). The growth rate of oceanic phytoplankton and the nitrogen isotope fractionation factor in nitrate assimilation (α) are negatively related: α decreases from 1.015 to 1.001 as μ increases, until isotope fractionation becomes negligibly small at the fastest growth rates (Wada and Hattori, 1991). Thus, phytoplankton $\delta^{15}N$ values differ depending on the depth at which phytoplankton grow. If zooplankton inhabit different depth ranges by season as shown by Takahashi *et al*. (2008), the $\delta^{15}N$ of zooplankton will also differ depending on the depth of sampling. Because we sampled using 150-m vertical tows in this study, we might have collected a mixed population from the surface layers to below the mixed layer within the same sampling net. We suspect that this dependence on sampling depth contributed to the observed variability of $\delta^{15}N$ for zooplankton.

Carbon and nitrogen trophic discrimination factor in the Oyashio waters

Wada *et al.* (1987) showed a clear linear relationship between the chemical parameter of isotopic ratios and the ecological parameter of TL. Mearns (1982) defined TL by the origin or food of small prey items identified from stomachs of predators from three Pacific Ocean food webs. He assumed that TL = 2.0 for calanoid copepods as phytoplankton feeders and that TL = 3.5 for chaetognaths preying on larval fish other than zooplankton (Mearns, 1982). We estimated inherent ratios of trophic fractionation of $\delta^{15}N$ ratios in the Oyashio based on linear regressions of observed $\delta^{15}N$ for zooplankton versus Mearns's assumed TLs.

In the Oyashio, we collected algae, *E. bungii*, *Neocalanus spp*., chaetognaths, and other

zooplankton in May. Therefore, the TL of algae was 1.0, and the TL of zooplankton was estimated by assuming a TL of 2.0 for *E. bungii*, which had the lowest value among the copepods, and a TL of 3.5 for chaetognaths (Mearns, 1982). The TL for other zooplankton were estimated based on their observed mean $\delta^{15}N$ values in May, assuming a linear relationship between $\delta^{15}N$ and TL. The enrichment of $\delta^{15}N$ per TL at the Oyashio (in May) was 3.5 ± 0.2‰ (mean ± S.E.; $r^2 = 0.91$, $p < 0.01$). Using the TL estimated from $\delta^{15}N$, the mean amplitude of $\delta^{13}C$ enrichment per TL was 0.9 ± 0.3‰ ($r^2 = 0.16$, $p < 0.05$). These values agreed with the 3 ± 1‰ per TL for $\delta^{15}N$ reported by DeNiro and Epstein (1981), Minagawa and Wada (1984), and Fry (1988), whereas $\delta^{13}C$ enrichment per TL was consistent with values of 0.7-1.5‰ per TL (average of 1‰) according to McConnaughey and McRoy (1979) and Rau *et al.* (1983).

$\delta^{15}N$ and $\delta^{13}C$ map for food chains in the Oyashio, WCR 86-B, Antarctic Ocean, and Gulf of Alaska

To test whether each ecosystem exhibits a distinct linear relationship between $\delta^{15}N$ and $\delta^{13}C$ ratios along the food chain, we combined our data with data from the Antarctic Ocean (AO; Wada *et al.*, 1987, Appendix Table VI) and the Gulf of Alaska (ALS; digitized and redrawn from Fig.1 of Kaeriyama, 2004). We examined the combined data for differences among the four oceanic regions. Fig. 6 combines data from Fig. 5 (the Oyashio [OY] and WCR 86-B) with those from the AO and ALS. Because different species were collected at each station, we aggregated the data by zooplankton group (salps, copepods, euphausiids, amphipods, and chaetognaths) and used the average values for each group in the analysis.

The $\delta^{15}N$–$\delta^{13}C$ map of each region indicates that the range of $\delta^{15}N$ was 1‰ to 15‰, and the range of $\delta^{13}C$ was generally -24‰ to -18‰, except for low $\delta^{13}C$ values in the AO

($\delta^{15}N$ = 0‰ to 11‰; $\delta^{13}C$ = -33‰ to -23‰). ANCOVA tests using the respective isotopic ratios as the response variable revealed no significant interactions between oceanic region and either $\delta^{15}N$ or $\delta^{13}C$ (Table III a-b; $p > 0.05$). A common $\delta^{15}N/\delta^{13}C$ slope was found, with only the intercept differing among the four oceanic regions (Table III c-d and linear regression line in Fig. 6; $p < 0.001$). For $\delta^{15}N$ as response variable, the regression yielded:

$\delta^{15}N = 1.53\ [\pm 0.25]\ \delta^{13}C + 40.9\ [\pm 5.6] +$ (respective constant for each region),

where the constant values for each region were OY: -1.71 ($p = 0.0075$), WCR 86-B: -1.61 ($p = 0.0253$), AO: 4.49 ($p = 0.0008$), and ALS: -1.17 ($p = 0.0173$).

Tukey's HSD post-hoc tests for differences among the slopes of the fitted lines revealed that AO differed significantly from OY, WCR 86B, and ALS (Fig. 7). Both the subarctic North Pacific and Antarctic Oceans are typical high-nutrient, low-chlorophyll (HNLC) regions where iron limits biological productivity (Martin and Fitzwater, 1988; Sohrin *et al.*, 2000; Bowei *et al.*, 2001; Tsuda *et al.*, 2003). The seasonal amplitudes of temperature and nitrate concentrations in the Gulf of Alaska are smaller than those in the Oyashio, but both regions are located in the same subarctic North Pacific gyre. In contrast, surface water temperature of the Antarctic Ocean is -0.5ºC to 2ºC (Wada *et al.*, 1987), and productivity is rather low despite high nitrate and silicate concentrations (e.g. Garcia *et al.*, 2010). In the AO, phytoplankton are limited by low light intensity and water temperature rather than by nutrient concentration, causing the low isotopic compositions (Wada *et al*., 1987; Wada and Hattori, 1991), which may partially explain the difference between zooplankton from AO and those from other regions.

Based on the ANCOVA tests, we obtained common slopes (parallel relationships) with different intercepts for the four oceanic regions. This common relationship may result

from the kinetic isotope effect inherent to the processes of amino acid synthesis as part of intermediary metabolism. It seems likely that the intercept of each parallel line depended on the isotopic composition of primary producers. The main metabolic pathways are common in almost all cells and organisms, and, in general, isotope ratios within organisms depend on the isotopic composition of reactants and on branch reactions within any *in vivo* metabolic pathway, such as carboxylation and some transamination reactions (Minagawa *et al.*, 1992). Additionally, Chikaraishi *et al*. (2009) proposed the use of the amino acid trophic level (ATL), with an emphasis on the occurrence of nitrogen isotope fractionation during amination and deamination processes. We therefore suspect that kinetic isotope effects are generated during the synthesis of amino acids (Macko *et al.,* 1986; Minagawa *et al.*, 1992). The composition of primary producers may also determine the intercept for each ecosystem on the $\delta^{15}N$–$\delta^{13}C$ map (Wada and Hattori, 1991). In fact, significant linear relationships between carbon and nitrogen isotope effects at the protein synthesis level were observed in the organs of a cormorant that consumed the same type of food for 23 years (Mizutani *et al.*, 1991). According to the simple mass balance calculation, the effect of this process does not alter the appearance of linearity on the isotopic map if the fractionation factor of the amino acid synthesis is constant throughout the food chain from primary producers to the animal at the highest TL. Consistent with this, the ANCOVA analyses suggested that the nitrogen and carbon isotope fractionation is constant irrespective of animal species in the present results. Considering these facts, the varying $\delta^{15}N/\delta^{13}C$ slopes might result from other factors that we have not analyzed, such as the availability of micronutrients for primary producers, *in vivo* amino acid metabolism, and NH_4^+ excretion systems including the urea cycle and processing of tricarboxylic acid cycle (TCA cycle). The magnitude of the fractionation factor may vary

depending upon the dynamic operation of energy production systems involving glycolysis, TCA cycle, and oxidative phosphorylation, which are associated with the synthesis of the carbon skeleton of amino acids (Pecquerie *et al.*, 2010).

The detrital food chain and the grazing food chain occur simultaneously in places such as lagoons, where $\delta^{13}C \approx 0$ (e.g. Wada *et al*., 1993). In this study, we did not directly observe the microbial loop. However, Kohzu *et al.* (1999) reported that wood-decomposing fungi completely exhaust wood nitrogen without nitrogen isotope fractionation under conditions of very low nitrogen availability (less than 0.07%). We therefore expect low $\Delta\delta^{15}N/\Delta\delta^{13}C$ slope values for food chains supported mostly by microbial loops. Based on our results, we also expect that the $\Delta\delta^{15}N/\Delta\delta^{13}C$ slope of each food chain may reflect ecological factors, such as the anabolic-catabolic ratio of primary producers, differences in season and/or habitat depth, mixed diets of heterotrophs, and differences in lipid content through the course of life cycles (Pecquerie *et al.*, 2010).

4. CONCLUSIONS

We investigated the relationship between nitrogen and carbon stable isotopes throughout the year in the Oyashio region of the western North Pacific. Isotopic ratios of higher TLs, such as predatory and/or long-lived zooplankton, varied little with season, while the isotopic ratios of short-lived zooplankton varied with season on our $\delta^{15}N$–$\delta^{13}C$ maps. Although it was not clarified from our original data alone, analysis using additional dataset from other two oceanic regions anticipates the existence of a common $\Delta\delta^{15}N/\Delta\delta^{13}C$ slope in oceanic food chains. We suspect that this common relationship most likely results from kinetic isotope effects in the processes of amino acid synthesis. However, we were only able to draw conclusions about regional differences in this

relationship by combining data from several sources. More extensive observations covering a range of trophic levels at each location will be needed to reach more detailed conclusions (e.g., concerning seasonal differences).

We expect lower $\Delta\delta^{15}N/\Delta\delta^{13}C$ slopes under oligotrophic conditions, increasing $\Delta\delta^{15}N/\Delta\delta^{13}C$ slopes as waters become more eutrophic, and very steep $\Delta\delta^{15}N/\Delta\delta^{13}C$ slopes in highly eutrophic coastal areas where $\Delta\delta^{13}C \approx 0$. However, the relationship between isotopic fractionation by branch reactions and trace metal availability remains poorly understood. Clarifying the role of trace metals or other controlling factors on isotopic fractionation *in vivo* is a promising topic for further research and would help to advance the understanding of the relationships among physical environments, physiology, and ecology.

Our results suggest several promising ways of further exploiting the potential of SI ratios as tracers of biogeochemical cycles in both terrestrial and aquatic environments. By analyzing isotopic ratios of zooplankton at life cycle levels, we can understand variation in C and N isotope ratios of higher TLs, such as fish and seabirds. The relationship between SI ratios of primary producers and their immediate consumers has already been researched extensively, but the relationship could be examined at higher trophic levels and elucidated more precisely by exploiting regional differences in SI ratios. For example, Minami and Ogi (1997) investigated the migratory dynamics and dietary changes of sooty shearwater in the Pacific using $\delta^{15}N$ and $\delta^{13}C$ of muscle tissue. Combining C and N SI ratios with other isotopes could allow for new developments in the field of traceability (Nakano, 2010). For example, Kennedy (2002) reconstructed the life histories of migratory fish using Sr isotopes in otoliths of Antarctic salmon. In recent years, higher TL ecosystem models have been used to investigate population sizes and seasonal migration

of fish using the results of lower trophic ecosystem models (e.g. Rose *et al.*, 2007; Okunishi *et al.*, 2009). SI data and models explicitly including SI could inform and validate such higher TL ecosystem models.

FUNDING

This work was supported by a Grant-in-Aid for Challenging Exploratory Research from the Japan Society for the Promotion of Science (22651007).

ACKNOWLEDGMENTS

We gratefully acknowledge Drs. Miwa Nakamachi, Hiromi Kasai, and Tsuneo Ono for providing the A-line field data (temperature, nitrate, and chlorophyll *a*). The authors also wish to thank Drs. Sanae Chiba, Taketo Hashioka, Masahito Shigemitsu, Kazutaka Takahashi, Yuji Okazaki, Akira Kuwata, Akio Ishida, and Chisato Yoshikawa for their helpful discussions and comments. We also thank to the scientists and crew members of the WK-09-03 and WK-09-07 cruises of R/V *Wakataka-maru*, the KY-09-05 cruise of R/V *Kaiyo-maru*, and the HK-09-10 cruise of R/V *Hokko-maru* with the Fisheries Research Agency (FRA) and Fisheries Agency.

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Table and Figure Legends

Table I

Nitrogen and carbon isotope ratios of phytoplankton and zooplankton collected at the A-line in the western North Pacific from March to October 2009. All species were collected from the upper 150 m. Values are mean ± SD. "*n*" indicates the number of samples analyzed (not population size), and "*n.d.*" indicates no data.

Table II

Nitrogen and carbon isotope ratios of zooplankton collected at Warm-core Ring 86-B in the western North Pacific from 1 to 25 September 1987. All species were collected within the upper 150 m. Values are mean ± SD. "*n*" indicates the number of samples analyzed (not population size), and "*n.d.*" indicates no data.

Table III

Results of ANCOVA on $\delta^{15}N$ and $\delta^{13}C$ of zooplankton in four oceanic regions: Oyashio, Warm-core Ring 86-B, Antarctic Ocean, and Gulf of Alaska. Significant effects at $p = 0.05$ are indicated in bold.

Fig. 1

Sampling area and location of the sampling stations of the A-line (a) and Warm-core Ring 86-B (b).

Fig. 2

Seasonal and latitudinal variation in the physical, chemical, and biological characteristics of the A-line of the western subarctic North Pacific. Averaged temperature (closed circles), nitrate (open circles), and chlorophyll *a* concentration (open triangles) in the mixed layer depth (MLD). The MLD is defined as the depth at which the potential density (σ_t) becomes 0.125 kg m^{-3} higher than the surface value (Aita *et al.*, 2007). Along the A-line, we distinguished between the Oyashio and Kuroshio waters based on the temperature at 100-m depth, where the isotherm is relatively stable, with little seasonal variation. Following Odate (1994), we classified water masses with temperatures (at 100

m; symbol +) below 5ºC as the Oyashio. Gray bar shows water masses of the Oyashio waters (OW). The symbol ☆ represent plankton sampling stations.

Fig. 3

Distribution of (a) nitrogen and (b) carbon isotope ratios of algae and dominant species of zooplankton collected from the A-line in the western subarctic North Pacific.

Fig. 4

Relationship between $\delta^{13}C$ and $\delta^{15}N$ values for zooplankton at the A-line in the Oyashio waters from March to October. Solid lines are derived from linear regressions of each month. Numbers in parentheses indicate the number of samples used for the analyses. The slopes of the lines in March, May, July, and October were 1.23, 0.61, 1.39, and 1.23, respectively. The correlation coefficient, r^2, for mean $\delta^{13}C$ versus $\delta^{15}N$ was 0.62 for March, 0.20 for May, 0.40 for July, and 0.27 for October. The symbol ● represents copepods; □, euphausiids; +, amphipods; ○, chaetognaths.

Fig. 5

Relationship between $\delta^{13}C$ and $\delta^{15}N$ values for zooplankton aggregated by group from the Oyashio waters (annual means, Table 1, shown in gray) and Warm-core Ring 86-B (from Table 2, shown in black). Solid lines are derived from linear regressions.

Fig. 6

Relationship between $\delta^{13}C$ and $\delta^{15}N$ values for zooplankton and fish from four oceanic regions: Oyashio: OY (○), Warm-core Ring 86-B: WCR 86-B (*), Antarctic Ocean: AO (●; taken from Wada *et al.*, 1987), and the Gulf of Alaska: GA (Δ; mean data redrawn from Kaeriyama, 2004). Symbols represent means ± SD, and solid lines are fits for each

region from ANCOVAs.

Fig. 7

Averaged $\delta^{15}N$ and $\delta^{13}C$ of samples from four oceanic regions (least mean squares fit ± SE): Oyashio (OY), Warm-core Ring 86-B (WCR 86-B), Antarctic Ocean (AO), and Gulf of Alaska (ALS). Within each panel, numbers with the same letter are not significantly different according to the Tukey's HSD post-hoc test at $\alpha = 0.05$ applied to the results of ANCOVAs (Table III c-d).

1 **SUPPLEMETARY DATA**

2

3 **Table IV Oyashio region at A-line**

4 *All samples were collected upper 150m. "*n*" indicates number of population size.

Samples	Stn.	Date	Location		$\delta^{15}N$	$\delta^{15}C$	*n*
		yyyy/mm/dd	Lat.	Lon.	(‰)	(‰)	
phytoplankton	A2	2009/05/03	42º67N	144 º91E	1.8	-22.2	
	A2.5	2009/05/03	42º58N	144 º96E	1.7	-21.6	
	A3	2009/05/03	42º50N	145 º01E	3.2	-21.3	
	A5	2009/05/04	41º99N	145 º24E	3.1	-21.9	
Eucalanus bungii	A2	2009/05/03	42º67N	144 º91E	5.2	-20.6	30
	A2.5	2009/05/03	42º58N	144 º96E	4.9	-20.7	23
	A3	2009/05/03	42º50N	145 º01E	5.3	-19.8	30
	A4.5	2009/05/04	42º12N	145 º18E	5.1	-22.4	20
	A5	2009/05/04	41º99N	145 º24E	4.6	-20.6	48
Eucalanus bungii	A2	2009/05/03	42º67N	144 º91E	5.2	-20.6	30
	A2.5	2009/05/03	42º58N	144 º96E	4.9	-20.7	23
	A3	2009/05/03	42º50N	145 º01E	5.3	-19.8	30
	A4.5	2009/05/04	42º12N	145 º18E	5.1	-22.4	20
	A5	2009/05/04	41º99N	145 º24E	4.6	-20.6	48
Neocalanus plumchrus	A5	2009/05/04	42º99N	145 º24E	7.4	-17.8	26
	A2	2009/07/14	42º40N	144 º55E	5.9	-20.7	40
	A2.5	2009/07/12	42º34N	144 º57E	6.3	-20.3	46
	A3	2009/07/12	42º30N	145 º00E	10.0	-18.6	14
	A3.5	2009/07/12	42º20N	145 º04E	9.1	-19.2	20
	A4	2009/07/12	42º15N	145 º07E	5.7	-24.4	30
	A4.5	2009/07/11	42º07N	145 º11E	6.7	-20.2	22
	A11	2009/07/16	40º29N	146 º00E	7.6	-19.3	9
	A13	2009/07/16	39º59N	146 º14E	7.6	-20.3	88

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1 **Table IV Oyashio region at A-line (continued)**

Samples	Stn.	Date	Location		$\delta^{15}N$	$\delta^{15}C$	*n*
		yyyy/mm/dd	Lat.	Lon.	(‰)	(‰)	
Neocalanus cristatus	A4	2009/03/05	42º15N	145º07E	6.1	-22.8	4
	A5	2009/03/05	42º00N	145 º15E	7.8	-21.0	1
	A5	2009/03/05	42º00N	145 º15E	6.1	-23.9	1
	A2	2009/05/03	42º67N	144 º91E	6.2	-21.8	2
	A3	2009/05/03	42º50N	145 º01E	6.9	-23.0	7
	A3.5	2009/05/03	42º50N	144 º08E	5.8	-23.7	55
	A4	2009/05/03	42º24N	145 º12E	5.9	-24.0	20
	A4.5	2009/05/04	42º12N	145 º18E	6.8	-22.4	19
	A5	2009/05/04	41º99N	145 º24E	6.9	-22.1	34
	A2	2009/07/14	42º40N	144 º55E	4.7	-22.5	11
	A2.5	2009/07/12	42º34N	144 º57E	5.9	-19.2	12
	A3	2009/07/12	42º30N	145 º00E	7.1	-19.5	8
	A3.5	2009/07/12	42º20N	145 º04E	6.4	-19.4	8
	A4	2009/07/12	42º15N	145 º07E	7.3	-19.6	9
	A4.5	2009/07/11	42º07N	145 º11E	5.4	-20.6	9
	A11	2009/07/16	40º29N	146 º00E	5.1	-22.1	10
	A13	2009/07/16	39º59N	146 º14E	6.2	-21.2	34
	A15	2009/07/16	39º29N	146 º29E	5.8	-19.6	5
Neocalanus flimingeri	A2	2009/05/03	42º67N	144 º91E	8.4	-20.6	32
	A2.5	2009/05/03	42º58N	144 º96E	8.1	-20.3	7
	A3	2009/05/03	42º50N	145 º01E	9.0	-21.2	20
	A3.5	2009/05/03	42º50N	144 º08E	9.2	-21.3	10
	A4.5	2009/05/04	42º12N	145 º18E	10.2	-22.0	8
	A5	2009/05/04	41º99N	145 º24E	8.2	-19.7	22
Euphausiids	A2	2009/03/04	42º39N	144 º54E	7.0	-20.6	4
	A2	2009/05/03	42º67N	144 º91E	7.7	-21.5	1
	A3	2009/05/03	42º50N	145 º01E	8.7	-22.1	3
	A3.5	2009/05/03	42º50N	144 º08E	8.2	-21.3	5
	A4	2009/05/03	42º24N	145 º12E	8.1	-21.5	6

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1 **Table IV Oyashio region at A-line (continued)**

Samples	Stn.	Date	Location		$\delta^{15}N$	$\delta^{15}C$	*n*
		yyyy/mm/dd	Lat.	Lon.	(‰)	(‰)	
Euphausiids	A4.5	2009/05/04	42º12N	145 º18E	7.9	-21.6	4
	A5	2009/05/04	41º99N	145 º24E	7.1	-21.7	2
	A2	2009/07/14	42º40N	144 º55E	7.4	-20.5	2
	A3	2009/07/12	42º30N	145 º00E	6.2	-19.8	3
	A4	2009/07/12	42º15N	145 º07E	7.7	-19.8	2
	A4.5	2009/07/11	42º07N	145 º11E	9.3	-18.5	18
	A13	2009/07/16	39º59N	146 º14E	6.8	-20.2	10
	A3	2009/10/11	42º29N	145 º00E	7.7	-20.9	40
	A3.5	2009/10/12	42º20N	145 º04E	6.9	-20.4	7
	A4	2009/10/12	42º25N	145 º07E	7.5	-20.7	6
	A7	2009/10/13	41º30N	145 º30E	7.9	-19.7	1
Amphipods	A2	2009/03/04	42º39N	144 º54E	11.3	-20.9	4
	A3	2009/03/04	42º29N	144º59E	10.4	-19.8	26
	A4	2009/03/05	42º15N	145º07E	9.5	-20.5	3
	A4	2009/05/03	42º24N	145 º12E	10.3	-21.0	9
	A4	2009/05/03	42º24N	145 º12E	7.4	-21.2	4
	A5	2009/05/04	41º99N	145 º24E	9.0	-19.0	1
	A2	2009/07/14	42º40N	144 º55E	6.0	-19.9	3
	A3.5	2009/07/12	42º20N	145 º04E	8.8	-18.8	4
	A4	2009/07/12	42º15N	145 º07E	7.1	-20.1	2
	A4.5	2009/07/11	42º07N	145 º11E	7.0	-19.3	4
	A13	2009/07/16	39º59N	146 º14E	7.5	-19.8	7
	A3	2009/10/11	42º29N	145 º00E	7.0	-19.7	5
	A3.5	2009/10/12	42º20N	145 º04E	9.0	-19.8	13
	A7	2009/10/13	41º30N	145 º30E	7.2	-19.7	5
	A15	2009/10/14	39º30N	146 º29E	10.3	-19.7	8
Chaetognath	A2	2009/03/04	42º39N	144 º54E	12.6	-19.9	3
	A3	2009/03/04	42º29N	144º59E	10.1	-20.0	1
	A4	2009/03/05	42º15N	145º07E	11.2	-21.4	14
	A2	2009/05/03	42º67N	144 º91E	11.4	-17.8	1
	A3	2009/05/03	42º50N	145 º01E	12.1	-19.1	5
	A3.5	2009/05/03	42º50N	144 º08E	10.7	-19.2	6
	A4	2009/05/03	42º24N	145 º12E	9.3	-21.4	4
	A5	2009/05/04	41º99N	145 º24E	11.1	-20.1	15
	A2	2009/05/03	42º67N	144 º91E	11.4	-17.8	1

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1 **Table IV Oyashio region at A-line (continued)**

Samples	Stn.	Date	Location		$\delta^{15}N$	$\delta^{15}C$	*n*
		yyyy/mm/dd	Lat.	Lon.	(‰)	(‰)	
Chaetognath	A2	2009/07/14	42º40N	144 º55E	10.4	-19.8	7
	A2.5	2009/07/12	42º34N	144 º57E	10.6	-18.7	11
	A3	2009/07/12	42º30N	145 º00E	10.3	-18.9	7
	A3.5	2009/07/12	42º20N	145 º04E	11.4	-18.3	4
	A4	2009/07/12	42º15N	145 º07E	12.3	-20.2	7
	A4.5	2009/07/11	42º07N	145 º11E	10.9	-19.4	26
	A11	2009/07/16	40º29N	146 º00E	10.3	-19.4	18
	A13	2009/07/16	39º59N	146 º14E	10.1	-19.5	26
	A15	2009/07/16	39º29N	146 º29E	10.0	-19.7	13
	A4	2009/10/12	42º15N	145 º07E	10.6	-19.6	4
	A4.5	2009/10/12	42º07N	145 º11E	11.1	-19.3	8
	A5	2009/10/12	41º59N	145 º13E	11.1	-18.8	8
	A7	2009/10/13	41º30N	145 º30E	10.0	-19.1	4

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1 **Table V Warm Core Ring (WCR) 86-B**

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Samples	Stn.	Date	Location		$\delta^{15}N$	$\delta^{15}C$	*n*
		yyyy/mm/dd	Lat.	Lon.	(‰)	(‰)	
Thalia democratica	6-C	1987/09/04	38º59N	144 º56E	6.4	-22.7	
	6-C	1987/09/04	38º59N	144 º56E	5.3	-22.1	
	18	1987/09/07	39º40N	144 º59E	6.5	-22.8	
	8-1	1987/09/05	40º06N	145 º08E	6.5	-22.5	
Salpa fusiformis	8-1	1987/09/05	40º06N	145 º08E	5.5	-22.1	
Thetis vagina	6-C	1987/09/04	38º59N	144 º56E	6.6	-21.9	
	18	1987/09/07	39º40N	144 º59E	5.5	-21.9	
Pseudocalanus sp.	18	1987/09/07	39º40N	144 º59E	8.8	-19.7	
Pareuchaeta japonica	18	1987/09/07	39º40N	144 º59E	8.4	-20.2	
Brachycelus latypes	6-C	1987/09/04	38º59N	144 º56E	10.3	-18.6	
Themisto japonica	6-C	1987/09/04	38º59N	144 º56E	9.1	-20.4	
Sagitta hexaptera	6-C	1987/09/04	38º59N	144 º56E	8.3	-19.2	

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1 **Table VI Antarctic Ocean (Wada *et al.*, 1987)**

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Samples	Stn.	Date yyyy/mm/dd	Location Lat.	Lon.	$\delta^{15}N$ (‰)	$\delta^{15}C$ (‰)
Salpa thompsoni	3’	1984/01/13	61º32S	150 º26E	1.8	-28.1
Parathemisto gaudichaudi	6	1984/01/21	60º00S	116 º02E	1.8	-27.1
Euphausia superba	5	1984/01/19	65º03S	118 º03E	2.7	-29.3
	PI3-2	1984/01/17	64º38S	127 º13E	1.0	-28.1
Euphausia triacantha	3’	1984/01/13	61º32S	150 º26E	3.1	-29.1
Euphausiids		1983/01/15	59º25S	43 º09W	4.2	-25.7
.		1983/01/12	59º40S	43 º13W	6.0	-26.0
Sagitta maxima	3'	1984/01/13	61º32S	150 º26E	5.6	-26.1
Coelenterata	6	1984/01/21	60º03S	116 º04E	6.6	-24.6
Polychaeta	6	1984/01/21	60º00S	116 º02E	5.3	-27.1
	3’	1984/01/13	61º32S	150 º26E	4.8	-26.7
Kondakoria longimama		1983/01/14	59º34S	43 º16W	7.0	-26.1
		1983/01/16	59º49S	43 º47W	7.2	-24.5
		1983/01/26	59º57S	44 º30W	6.5	-25.5
Electrona Antarctica	3’	1984/01/13	61º32S	150 º26E	7.5	-27.1
Notolepis coasti	3’	1984/01/13	61º32S	150 º26E	7.1	-25.8
Trematomus bernacchir		1982/01/25			10.4	-23.4

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8

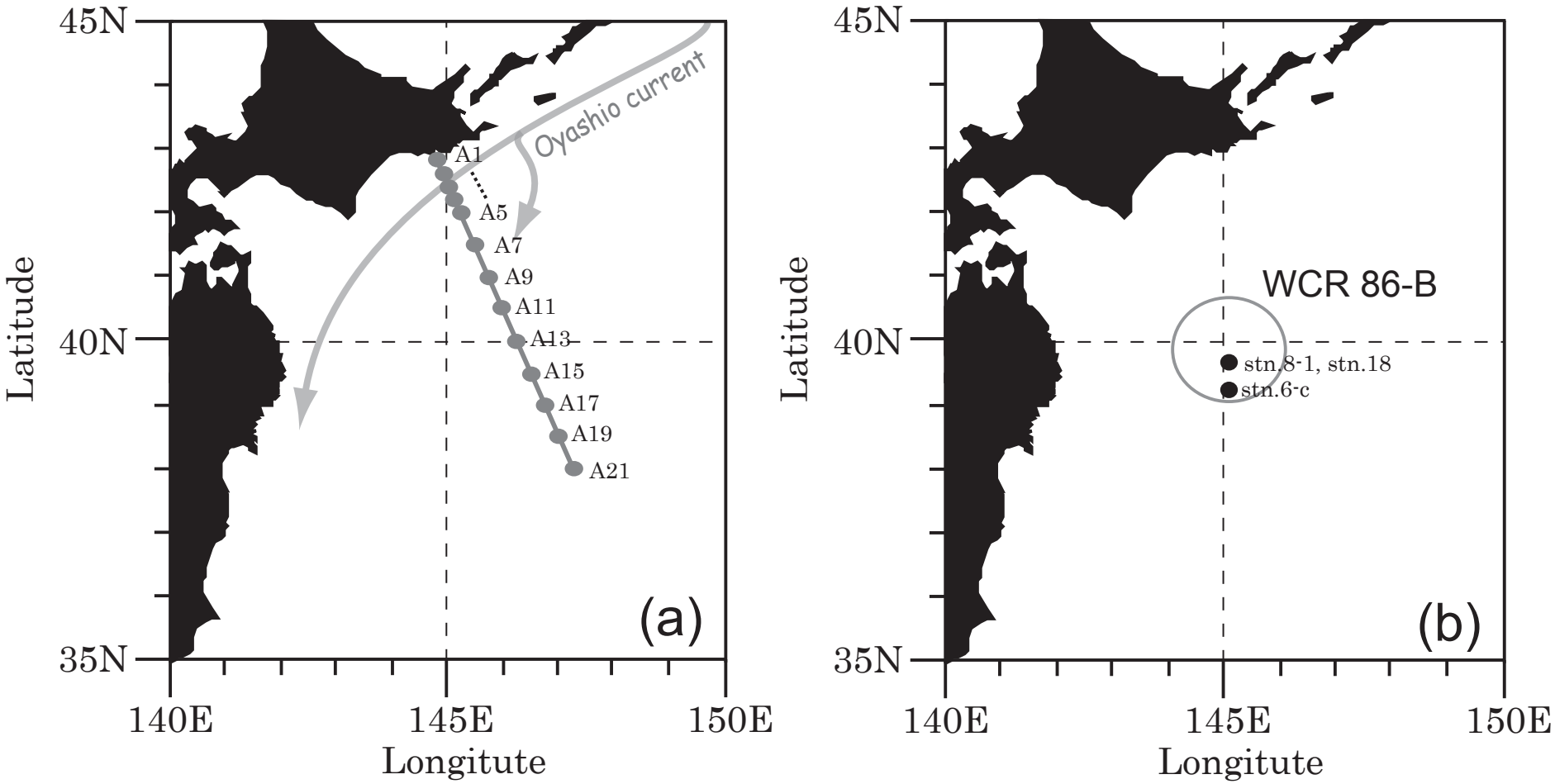
45N
40N
35N
140E
145E
150E
Latitude
Longitute
Oyashio current
A1
A5
A7
A9
A11
A13
A15
A17
A19
A21
(a)
WCR 86-B
stn.8-1, stn.18
stn.6-c
(b)

Fig. 1

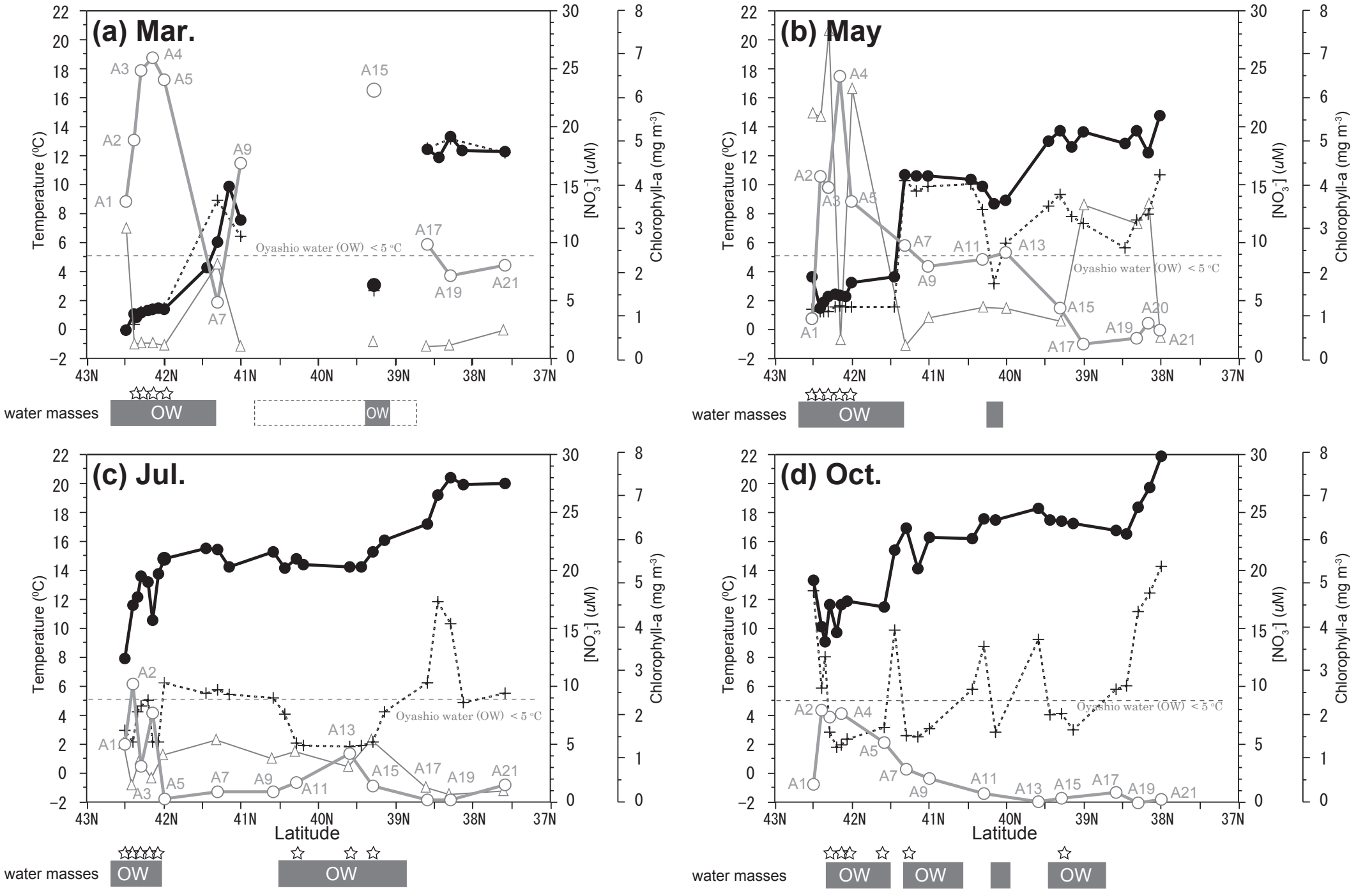
(a) Mar.
(b) May
(c) Jul.
(d) Oct.
Temperature (°C)
[NO3-] (uM)
Chlorophyll-a (mg m-3)
Latitude
Oyashio water (OW) < 5 °C
water masses
OW
averaged temperature in the MLD
temperature at 100m depth
averaged nitrate concentration in the MLD
averaged Chl-a concentration in the MLD
samplling stations

Figure 2

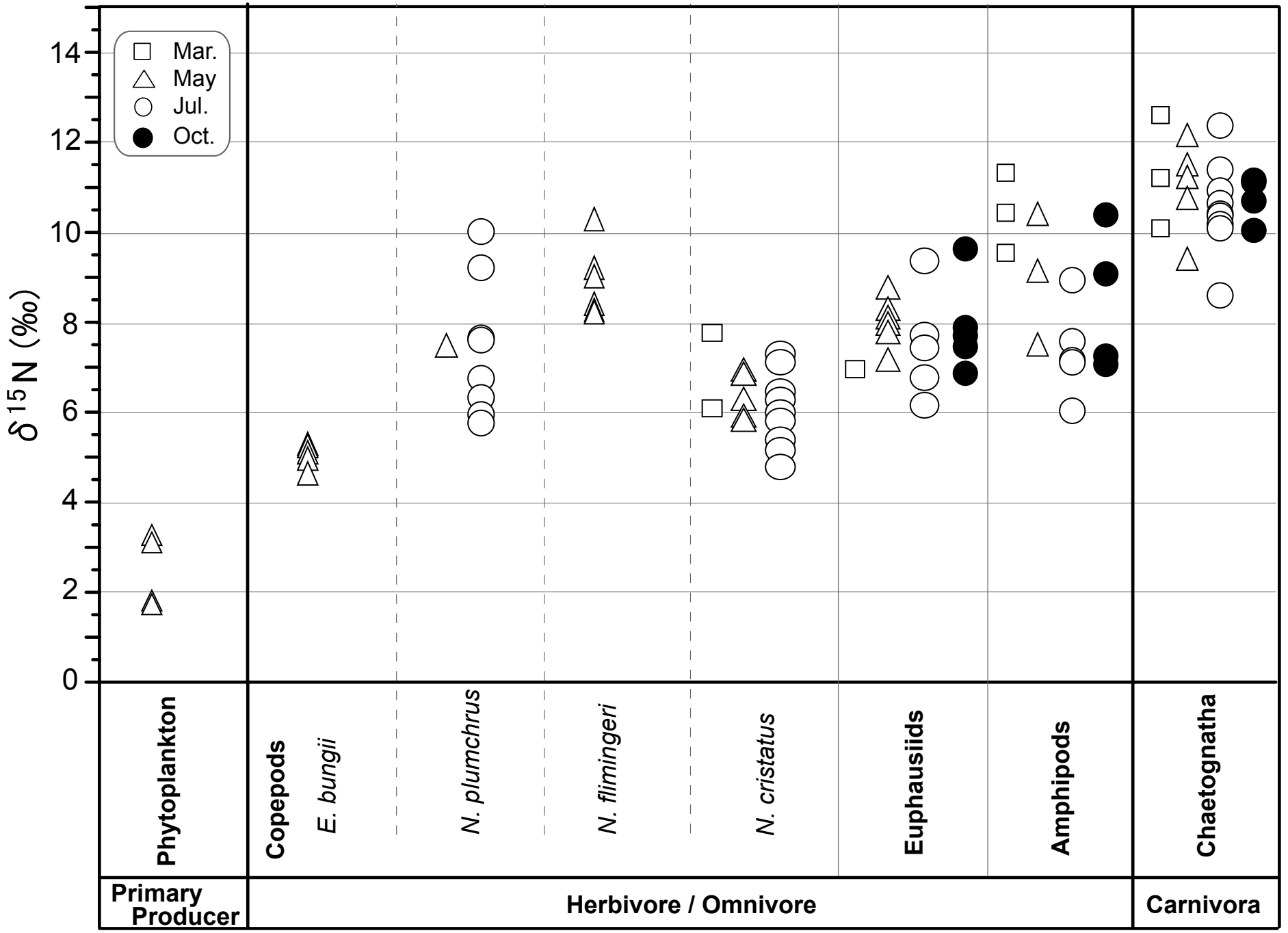
Mar.
May
Jul.
Oct.
δ15N (‰)
0
2
4
6
8
10
12
14
Phytoplankton
Copepods
E. bungii
N. plumchrus
N. flimingeri
N. cristatus
Euphausiids
Amphipods
Chaetognatha
Primary Producer
Herbivore / Omnivore
Carnivora

Fig. 3a

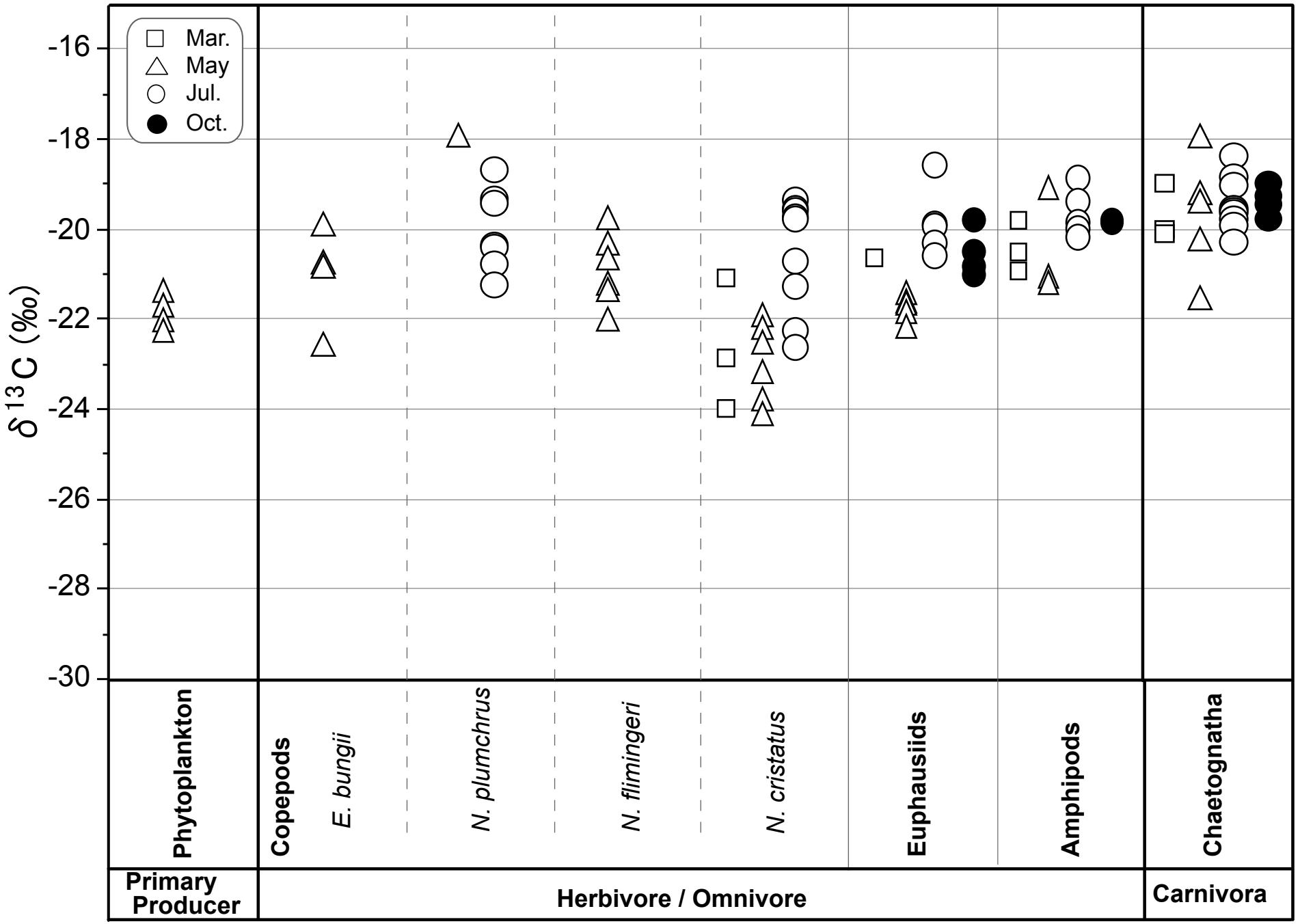
Mar.
May
Jul.
Oct.
-16
-18
-20
-22
-24
-26
-28
-30
δ13C (‰)
Phytoplankton
Copepods
E. bungii
N. plumchrus
N. flimingeri
N. cristatus
Euphausiids
Amphipods
Chaetognatha
Primary Producer
Herbivore / Omnivore
Carnivora

Fig. 3b

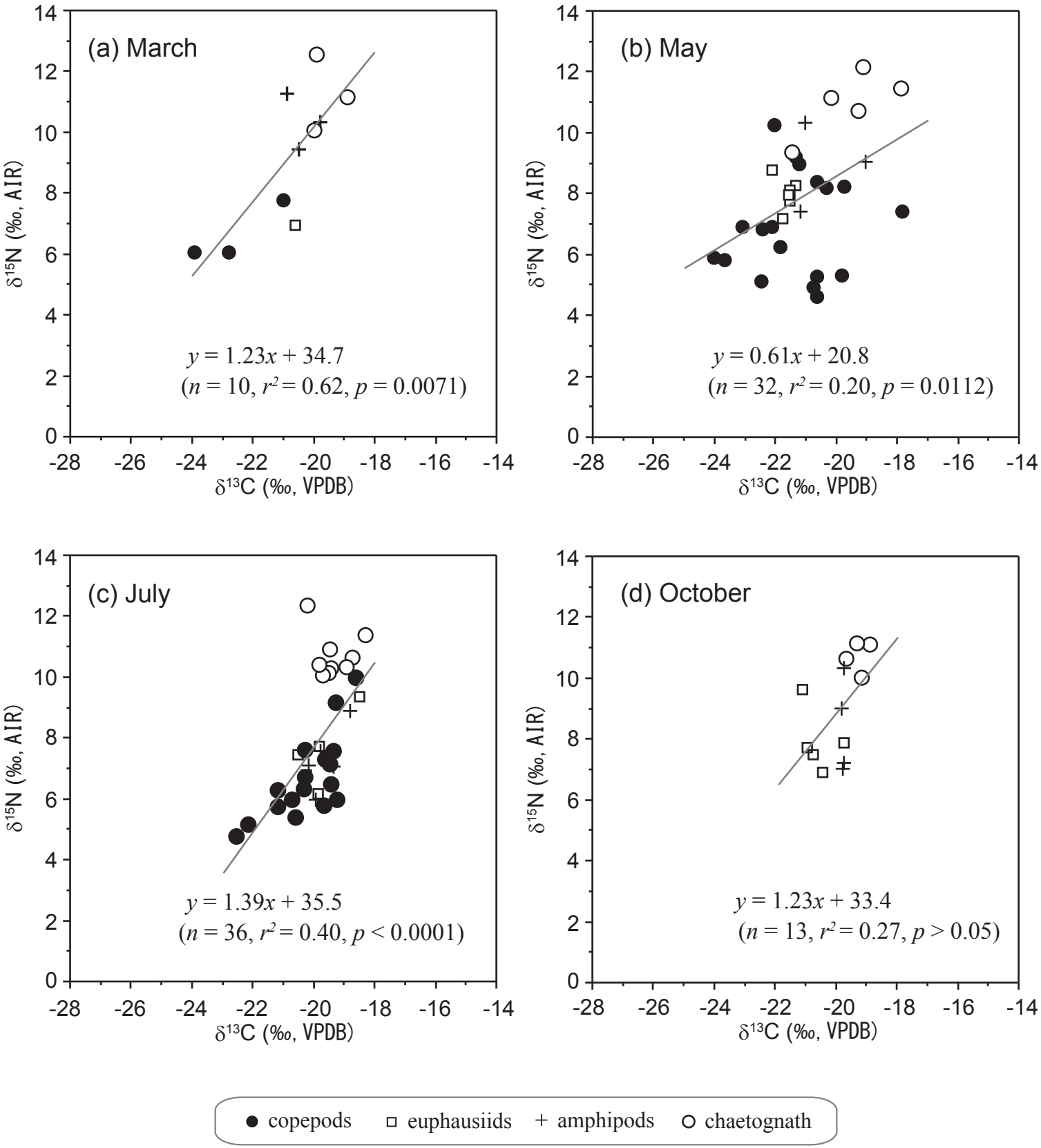
(a) March
y = 1.23x + 34.7
(n = 10, r² = 0.62, p = 0.0071)
(b) May
y = 0.61x + 20.8
(n = 32, r² = 0.20, p = 0.0112)
(c) July
y = 1.39x + 35.5
(n = 36, r² = 0.40, p < 0.0001)
(d) October
y = 1.23x + 33.4
(n = 13, r² = 0.27, p > 0.05)
δ15N (‰, AIR)
δ13C (‰, VPDB)
copepods
euphausiids
amphipods
chaetognath

Fig. 4

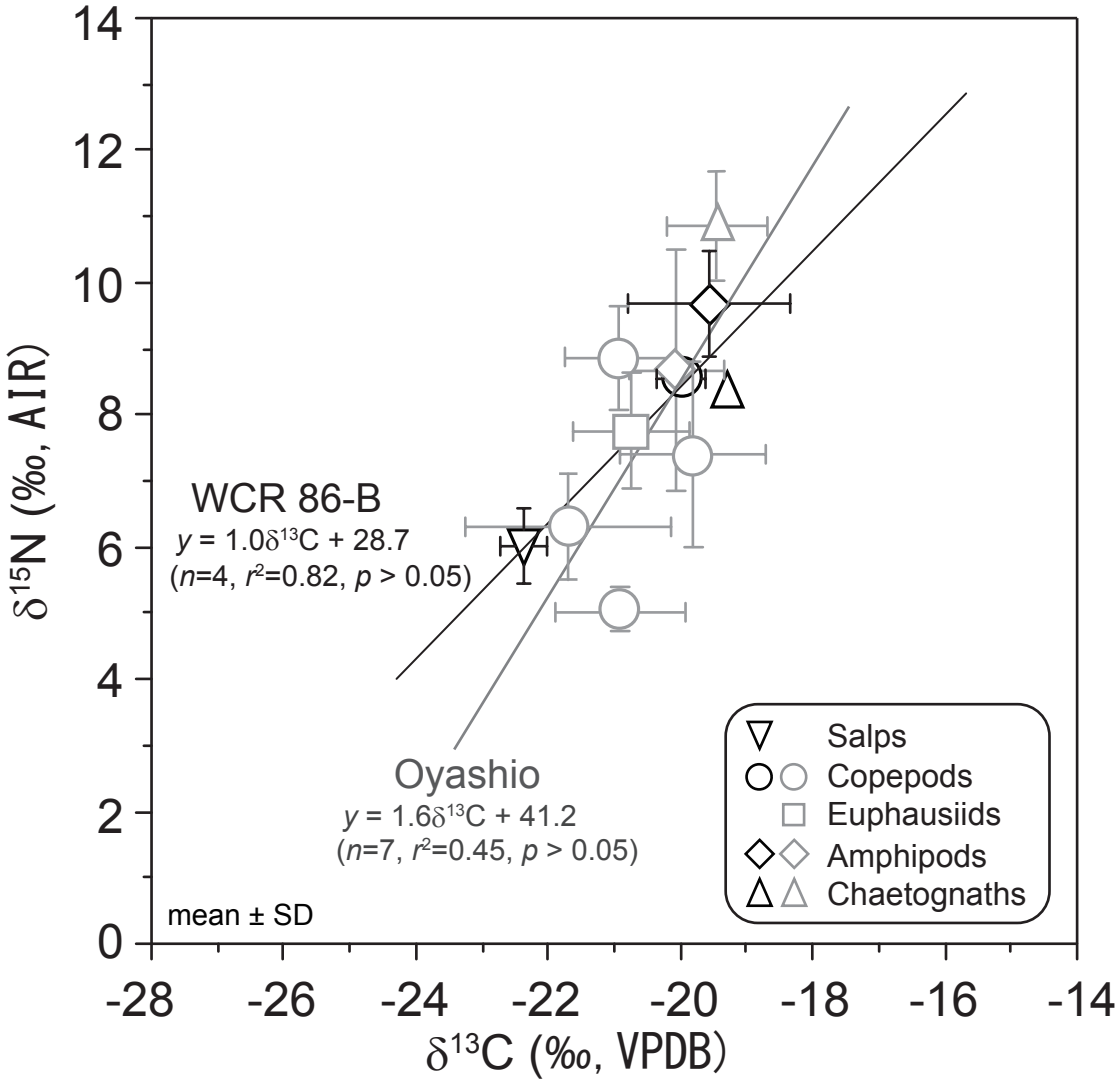
14
12
10
8
6
4
2
0
-28
-26
-24
-22
-20
-18
-16
-14
δ15N (‰, AIR)
δ13C (‰, VPDB)
WCR 86-B
y = 1.0δ13C + 28.7
(n=4, r2=0.82, p > 0.05)
Oyashio
y = 1.6δ13C + 41.2
(n=7, r2=0.45, p > 0.05)
mean ± SD
Salps
Copepods
Euphausiids
Amphipods
Chaetognaths

Fig. 5

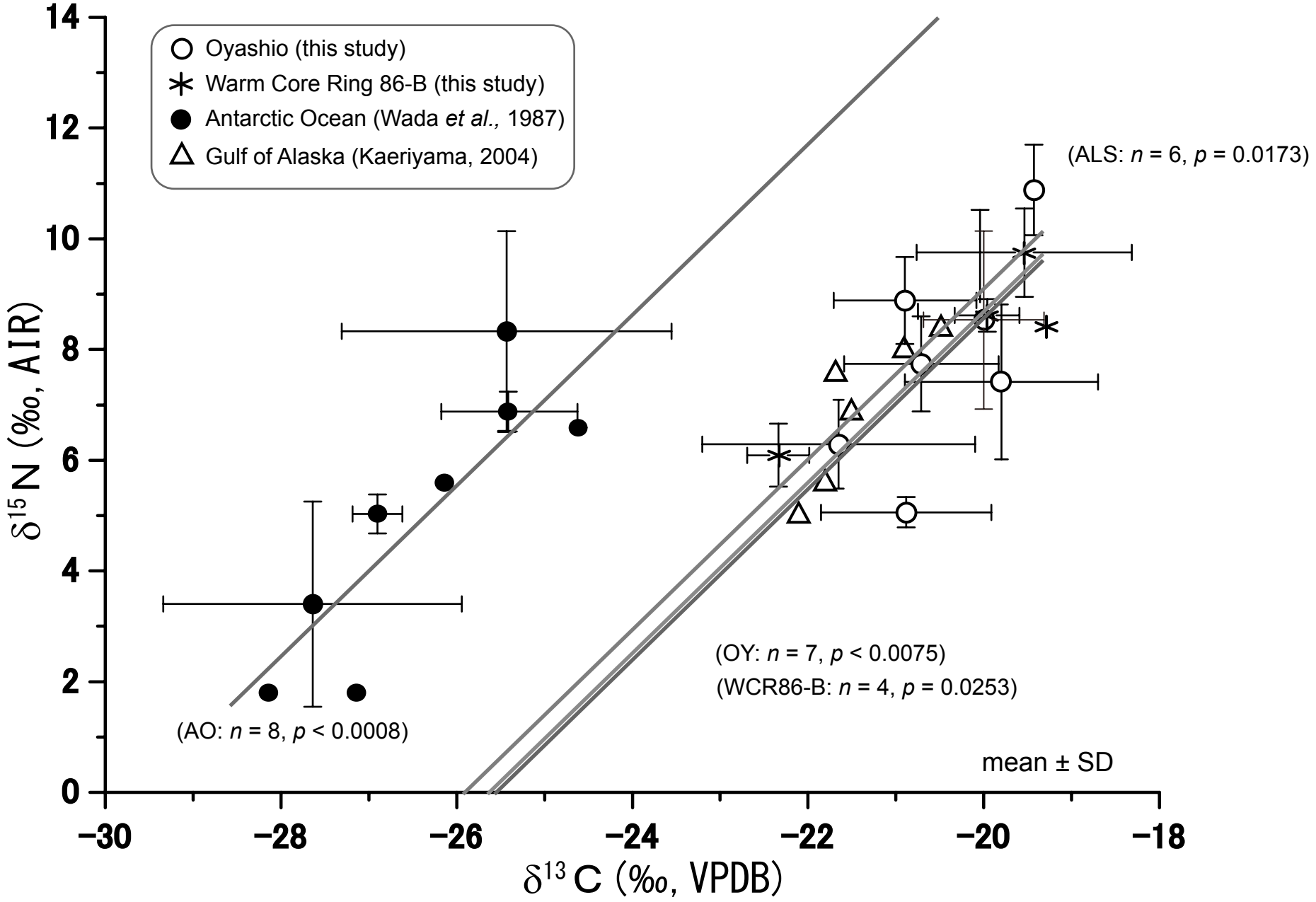
Oyashio (this study)
Warm Core Ring 86-B (this study)
Antarctic Ocean (Wada et al., 1987)
Gulf of Alaska (Kaeriyama, 2004)
(ALS: n = 6, p = 0.0173)
(OY: n = 7, p < 0.0075)
(WCR86-B: n = 4, p = 0.0253)
(AO: n = 8, p < 0.0008)
mean ± SD
δ15N (‰, AIR)
δ13C (‰, VPDB)
14
12
10
8
6
4
2
0
−30
−28
−26
−24
−22
−20
−18

Fig. 6

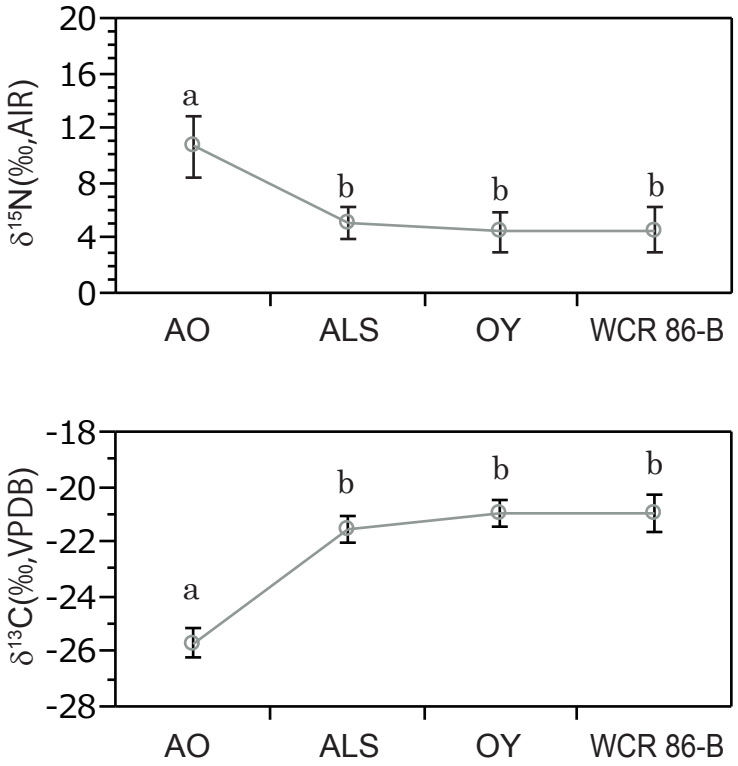
20
16
12
8
4
0
δ15N(‰,AIR)
a
b
b
b
AO
ALS
OY
WCR 86-B
-18
-20
-22
-24
-26
-28
δ13C(‰,VPDB)
a
b
b
b
AO
ALS
OY
WCR 86-B

Fig. 7