Ecological aspects of carbon and nitrogen isotope ratios of cyanobacteria

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Abstract: Variation in the δ13C of a cultured marine cyanobacterium (Agmenellum quadruplicatum, strain PR-6) is described with an emphasis on the relationships between growth rate, pCO2, and δ13C. An average nitrogen isotope fractionation of 1.0017 was obtained during N2 fixation by cultured marine cyanobacteria. This result suggests that nitrogen supply via N2 fixation may be characterized by a low δ15N, down to ca. −2‰ in marine environments, where the average δ15N is higher (at ca. 6‰) for major inorganic nitrogenous compounds such as nitrate. The natural abundances of nitrogen and carbon isotope ratios for cyanobacteria collected from the McMurdo Dry Valleys, Antarctica, were characterized by extremely low δ15N and widely ranging δ13C. Summarizing these and other data obtained, an isotopic map of cyanobacteria and other plankton in aquatic ecosystems was constructed and the ecological implications are discussed. Our results suggest that the δ13C of marine phytoplankton is positively correlated with sea surface temperature and can be a useful parameter for estimating in situ growth rates in the open ocean.

Key words: Antarctica, C and N isotope ratios, cyanobacteria, marine plankton, N2 fixation

Introduction

Stable isotope studies can be useful for understanding nutrient cycling, as well as the physiological nature of microorganisms in aquatic ecosystems. In photosynthetic organisms, carbon isotope fractionation is generally recognized to occur during photosynthetic carbon fixation. In his comprehensive review, O’Leary (1981) demonstrated that the carbon isotope effect in the C3 pathway is large during the carboxylation step. However, the diffusion of CO2 into leaves is partially rate-limiting and this serves to reduce in vivo fractionation, suggesting a possible correlation between the carbon isotope effect and the growth physiology of marine phytoplankton. The CO2 content of supplied gas has a major effect on the δ13C of microalgae (Mizutani & Wada 1982). δ13C in marine plankton is thought to range from −9 to −30‰ (Wada & Hattori 1991), depending on temperature, pH (which affects the HCO3−-CO2 isotope exchange equilibrium), and carbon availability. Based on latitudinal, hemispheric, and polar discrepancies in plankton δ13C, Rau et al. (1982) and Goerlcke & Fry (1994) emphasized that geographic differences in kinetic isotope effects associated with plankton biosynthesis and metabolism were important factors causing latitudinal variation in plankton carbon isotope abundance.

In a continuous culture of Chlamydomonas reinhardtii, Takahashi et al. (1991) found a linear relationship between the growth rate constant and the carbon isotope fractionation factor under light- and nitrogen-limited conditions. The same trend was also reported for nitrate-uptake processes in the marine diatom Phaeodactylum tricornutum (Wada & Hattori 1978). Because information on the growth physiology of phytoplankton and epibenthic algae can likely be obtained by measuring their C and N stable isotope ratios, Wada & Hattori (1991) created a δ15N–δ13C map of marine phytoplankton.

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In the open ocean, the nitrogen isotope ratios of plankton are closely correlated with the chemical forms of inorganic nitrogen used for primary production. The level of $\delta^{15}N$ in *Trichodesmium* sp. is low compared to those of plankton commonly found in other areas, supporting the idea that nitrogen is supplied to this community via molecular nitrogen fixation (Minagawa & Wada 1986, Saimo & Hattori 1987, Carpenter et al. 1997). The aerobic nitrogen fixation ability of *Trichodesmium* has been confirmed using isolated strains (Ohki et al. 1986, 1988, Chen et al. 1996, Ohki 2008, Finzi-Hart et al. 2009). However, nitrogen fixation sometimes occurs at low rates relative to the total nitrogen budget (Ohki et al. 1991, Capone et al. 1997). Unicellular diazotrophic cyanobacteria contribute significantly to nitrogen cycling in the ocean (Zehr et al. 2001), although $\delta^{15}N$ data for these unicellular species are not yet available.

Cyanobacteria are distributed across a wide range of physicochemical conditions, such as pH, redox potential, and temperature, and play important roles as primary producers in a variety of natural ecosystems. In the McMurdo Dry Valleys, Victoria Land, Antarctica, there are a variety of saline lakes and ponds that host undescribed types of cyanobacteria with the lowest known $\delta^{15}N$ values in the biosphere. In the saline lakes and ponds the cyanobacteria use nitrate supplied via precipitation. Because of the very low humidity in the McMurdo Dry Valleys, the precipitated nitrate is present as a powdered crystal of NaNO$_3$, which is the nitrogen source used for growth by the cyanobacteria in the saline lakes and ponds in this area. The $\delta^{15}N$ of sodium nitrate (NaNO$_3$), which can be as low as $-20\%$, and nitrogen isotope fractionation during nitrate uptake are possible causes of these low $\delta^{15}N$ values (Wada et al. 1981, Matsumoto et al. 1987, Matsumoto et al. 1993). Hage et al. (2007) isotopically characterized coastal pond-derived organic matter in the McMurdo Dry Valleys. Recently, Hopkins et al. (2009) reported isotopic evidence for the provenance and turnover of organic carbon by soil microorganisms in the McMurdo Dry Valleys.

In this study, we investigated the effects of cyanobacterial growth rate on carbon isotope fractionation in a marine cyanobacterium (*Agmenellum quadruplicatum*; PR-6). Marine diazotrophs were also investigated to elucidate the role of nitrogen isotope fractionation in molecular nitrogen fixation by various kinds of marine cyanobacteria. Cyanobacteria collected from saline lakes and ponds in the McMurdo Dry Valleys, Antarctica, were also studied in detail, with an emphasis on cyanobacterial growth physiology. Finally, we summarized the characteristics of cyanobacteria on a $\delta^{15}N$ vs. $\delta^{13}C$ map and discuss some ecological implications of algal isotope ratios based on our data in combination with previously reported data (Minagawa & Wada 1986, Wada & Hattori 1991, Yoshioka et al. 1994, Aita et al. 2011).

### Materials and Methods

**Cultures**

*Agmenellum quadruplicatum*, strain PR-6 (*Synechococcus* sp. ATCC27264/PCC7002), was kept in the laboratory of one of the authors (Van Baalen) in an axenic form. The organism was grown in modified ASP-2 medium (Van Baalen, 1962) in test tubes (100 ml) or Erlenermyr flasks (1 L) with continuous bubbling of 1% CO$_2$ ($\delta^{13}C$ PDB=−36.64%) and illuminated with fluorescent lamps 10 cm from the growth apparatus (50–60 $\mu$mol photons m$^{-2}$ sec$^{-1}$). To control the growth rate, light intensity was altered using black mesh cloth to cover the test tubes. Growth of the liquid cultures was followed turbidimetrically and the specific growth rate constant ($\mu$) was estimated using the method of Kratz and Myers (1955), where $\mu=0.301$ corresponds to a generation time of 1.00 d.

Cells for isotopic studies were harvested at the late logarithmic phase or early stationary phase for test tube cultures. In large-scale cultures, cells were harvested at intervals and collected on Whatman type-C glass fiber filters preheated to 400°C for 5 h. Filters with residues were treated with 0.1 N HCl and stored in a refrigerator until mass spectrometric analysis.

**Diazotrophs**

*Trichodesmium* spp. NIBB1067 was isolated and cultured in a synthetic medium as described in Ohki & Fujita (1982) and Ohki et al. (1986). Five unicellular and one filamentous marine cyanobacteria isolated from the sea around Singapore (Ohki et al. 2008) and *Crocosphaera watsonii* WH8501 (Waterbury and Pippka 1989) provided by Drs. Waterbury and Dyhman of Woods Hole Oceanographic Institution, USA, were maintained in a liquid modified Aquil medium without combined nitrogen (Ohki et al. 1986). Two freshwater heterocystous cyanobacteria in sections IV and V (*Nostoc* sp. PCC7120 and *Fischerella spl1ATCC29114*, respectively) were cultured in BG$_6$ (Rippka & Herdman 1992), and used as references. Cultures were kept under fluorescent lights (daylight type) at 10 $\mu$mol photons m$^{-2}$ sec$^{-1}$ (12 : 12 L : D) at 26°C (Ohki et al. 2008). Several milligrams of cells were collected, centrifuged at 3,000 × g for 4 min at 4°C, and lyophilized for stable isotope measurements.

**Cyanobacteria from the McMurdo Dry Valleys, Antarctica**

Observations of the McMurdo Dry Valleys area during the 1980–1981 field season were reported in detail by Wada et al. (1984). Lake Vanda, several ponds in the Labyrinth, Lake Bonney, and other lakes were visited during the season. Epibenthic cyanobacteria were collected from unnamed saline ponds in the Labyrinth, near the terminus of the Wright Upper Glacier, and from Lake Vanda and its surroundings. Samples were also collected from the Taylor
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Valley (Fig. 1).

Samples were frozen and brought back to Japan. Preliminary results from these samples were reported by Wada et al. (1981). Sterol distribution in the saline ponds can be found in Matsumoto et al. (1982). The geochemical features of the McMurdo Dry Valley lakes and ponds were reviewed by Matsumoto (1993).

To identify cyanobacteria, small aliquots of freeze-dried samples (5–50 mg) were taken, and DNA was extracted using a FastDNA Spin kit (MP Biomedicals, Solen, OH, USA). Most of the 16S rRNA gene fragments were amplified using cyanobacteria-specific primers as follows: 8F (forward, Wilmotte et al. 1993) and PISTE-Cyano-R (reverse, CTCTGTGCCAAGGTATC, Harverkamp, unpublished data). The conditions for polymerase chain reaction (PCR) were the same as those used in Ohki et al. (2008). The PCR products were ligated into a pGEM-T vector (Promega, WI, USA), and transformed into Escherichia coli cells to construct clone libraries. Clones containing an insert were selected and sequenced after the plasmid was purified. Homology-based searches of the DNA Data Bank of Japan (DDBJ) were performed using the BLAST program on the DDBJ website (http://www.ddbj.nig.ac.jp/index-j.html).

Isotope analyses

The samples collected from Antarctica were analyzed using an RMU-6R mass spectrometer (Hitachi) fitted with a double collector for ratiometry. The cultured diazotrophs were measured by Flash 2000, CoFloIV, Delta V (Thermo Fisher Scientific) in SI Science Co., Ltd., Japan (Aita et al. 2011). The isotopic data are presented in terms of δ\(^{13}\)C as the per mil deviation relative to the PDB isotopic standard. For δ\(^{15}\)N, atmospheric nitrogen was used as a standard. The precisions of δ\(^{13}\)C and δ\(^{15}\)N measurements were within 0.2‰.

\[
\delta^{13}\text{C or } \delta^{15}\text{N (‰)} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 10^3
\] 

where \(R\) is \(^{13}\)C/\(^{12}\)C or \(^{15}\)N/\(^{14}\)N, respectively.

The \(\delta^{13}\)C values of PR-6 are reported relative to the CO\(_2\) used as the carbon source.

Carbon isotope discrimination (\(\Delta\delta^{13}\)C) is generally expressed as a difference in the \(\delta^{13}\)C value between source and product (Takahashi et al. 1991):

\[
\Delta\delta^{13}\text{C} = \frac{\delta^{13}\text{C (source)} - \delta^{13}\text{C (product)}}{1 + \delta^{13}\text{C (source)}} \times 1000
\]

Spontaneous discrimination factor at biomass \(\text{N}_1\) and \(\text{N}_2\)
in the flask cultures was estimated by the following isotope mass balance equation:

\[
\delta^{13}C_{\text{Nt1}} \times f + \delta^{13}C_{\text{Nt2}}(1-f) = \delta^{13}C_{\text{Nt2}}
\]

where \( f = \text{Nt}_1/\text{Nt}_2 \) and \( \Delta t = t_2 - t_1 \).

Equation (3) can be re-arranged to obtain \( \delta^{13}C_{\text{Nt2}} \), which is the instantaneous discrimination factor during the interval between \( t_1 \) and \( t_2 \). This was done for the FL-1 and FL-2 incubation series in Fig. 2.

**Results**

*Agmenellum quadruplicatum* (PR-6)

Cyanobacterial cells with different growth rates under different light intensities exhibited marked variation in \( \delta^{13}C \) values (Fig. 2). The \( \delta^{13}C \) of cells ranged from \(-19.1\) to \(-10.7\%\), relative to the CO₂ used as the substrate. These values correspond to discrimination factors of 19.8 and 11.1, respectively. The maximum discrimination factor was observed in cells grown under low light intensities with low growth rates. The variation in \( \delta^{13}C \) of the cells was divided into two groups: For one group there was a negative correlation with growth rate; that is, the \( \delta^{13}C \) of the cells decreased with decreasing growth rate. The other group, shown within the dotted circle in Fig. 2, which had rather low discrimination factors, were collected in the late logarithmic phase or early stationary phase. Under those conditions, cell numbers in the incubation tube were maximal, meaning that high photosynthetic activity was rapidly consuming dissolved carbon dioxide.

**Diazotrophs**

The nitrogen isotope ratios (\( \delta^{15}N \)) of cultured cyanobacteria during N₂ fixation ranged from \(-1.5\) to \(-3.0\%\) relative to atmospheric N₂, with an average value of \(-1.74\%\), corresponding to a fractionation factor of 1.0017 (Table 1). These values were constant regardless of algal species and were comparable to those obtained for other N₂-fixing microorganisms, including *Azotobacter*. Fractionation during biological molecular nitrogen fixation was almost identical among these organisms.

**The partial rDNA sequence of cyanobacteria in the McMurdo Dry Valleys, Antarctica**

Diazotrophic cyanobacteria closely related to the genera *Leptolyngbya*, *Oococillaria*, and *Phormidium* (N₂-fixing under anaerobic conditions, cf. Rippka & Herdman 1992) were detected by partial sequencing of PCR-amplified 16S rDNA using DNA extracted from the samples collected from Antarctic saline ponds and lakes in the McMurdo Dry Valleys.

**Natural C and N isotope abundances of cyanobacteria in McMurdo Dry Valleys**

The natural C and N abundances of cyanobacteria collected from saline ponds and lakes in McMurdo Dry Valleys were precisely measured. \( \delta^{15}N \) values divided samples into two groups with average values of \(-10\) and \(-50\%\), respectively (Fig. 3 and Appendix). According to Wada et al. (1981), the \( \delta^{15}N \) of soil nitrate in McMurdo Dry Valleys is quite low, from \(-24\) to \(-11\%\). This can be considered a result of the characteristic origins of nitrate associated with atmospheric precipitation, including the production of NO₃ by auroral activity. The former group (\( \delta^{15}N \) of \(-10\%\)) reflects source nitrate \( \delta^{15}N \), whereas the latter (low \( \delta^{15}N \)) group probably arose under conditions of high nitrate concentration and low light intensity, with a significant occurrence of nitrogen isotope fractionation (up to 1.04) during nitrate assimilation. In fact, the occurrence of a large nitrogen isotope fractionation was observed for several saline ponds in the Labyrinth (Table 2). On one hand, for low NO₃ concentrations down to 1 μM, no nitrogen isotope discrimination is expected during nitrate assimilation. Consequently the \( \delta^{15}N \) of cyanobacteria in such ponds becomes close to the \( \delta^{15}N \) of nitrate (\(-20\%\)) used for growth. However, the \( \delta^{13}C \) was highly variable, ranging from \(-25\) to \(-3\%\). High values resulted from the limitation of CO₂ supply during growth. Algal mats are well known to show
such high $\delta^{13}C$ values.

**Discussion**

Theoretical consideration of steady-state C/N kinetic isotope effects

Isotope fractionation during photosynthetic CO$_2$ fixation and nitrate assimilation can be described in the following way (Farquhar et al. 1989, Takahashi et al. 1991).

$$CO_2 \xrightarrow{F1} CO_2 \xrightarrow{F2} \text{Organic-C}$$

The first step is CO$_2$ transport into the cell through bidirectional passive diffusion ($F1$ and $F3$), and the next is assimilation ($F2$) into organic carbon by ribulose bisphosphate carboxylase (RuBPCase).

Under the steady state condition during photosynthetic carbon fixation, we obtain

$$\Delta \delta^{13}C = b + (a - b) \times F2/F1$$

(4)
where $a$ and $b$ are the discrimination factors (DF) associated with the processes of CO$_2$ diffusion ($F_1$) and carboxylation reaction ($F_2$), respectively (Takahashi et al. 1991). For this simple model, we can obtain DF of 4.4 and 30 for the diffusion process and carboxylation reaction, respectively. Then we obtain

$$\Delta \delta^{13}C = 30 + (4.4 -30) \times F_2/F_1$$

(5)

Here we predict that the quotient $F_2/F_1$ in the above equation is proportional to $\mu$, because $F_1$ seems to be independent of $\mu$ and kept constant under constant ambient $p$CO$_2$. Thus, a constant $F_1$ flux implies a negative relationship between $\Delta \delta^{13}C$ and either $F_2$ or $\mu$. In fact, during algal photosynthesis, $\Delta D$ has a negative relationship with the growth rate constant $\mu$ (Takahashi et al. 1991). The following equation was obtained by Takahashi et al. (1991) for Chlamydomonas reinhardtii Dangeard (IAM C-238):

$$\Delta \delta^{13}C = -35.4 \mu + 25.3 (r = -0.92)$$

(6)

The nitrogen isotopic fractionation factor is inversely correlated with the growth rate constant in the assimilation of nitrate by the marine diatom Phaeodactylum tricornutum. The nitrogen isotope fractionation factor is 1.001 at high growth rates of P. tricornutum, and goes up to 1.023 under light-limited conditions (Wada & Hattori 1978). In view of these facts, variation in cellular $\delta^{13}C$ was examined for the cyanobacterium PR-6.

*Agmenellum quadruplicatum* (PR-6)

The enzymatic carboxylation reaction is the primary factor contributing to carbon isotope fractionation during photosynthesis (Christeller et al. 1976, Estep et al. 1978). The temperature-dependent isotope exchange equilibrium between CO$_2$ and bicarbonate, $p$CO$_2$, CO$_2$ diffusion, and diffusion/carboxylation partitioning ($F_2/F_1$ in the eq. (5)) relating to the growth physiology of organisms are also principal components governing the magnitude of carbon isotope fractionation (O’Leary 1981). Of these factors, the growth physiological condition is most likely to cause fluctuation or deviation in the $^{13}C$ content of phytoplankton in the surface waters of the ocean. The variation in $\delta^{13}C$ in PR-6 in the present study also suggests that carbon isotope fractionation is regulated by the kinetics of photosynthetic carbon fixation pathways. The differential or instantaneous carbon isotope discrimination factor decreased with an increase in its growth rate, similar to Chlamydomonas reinhardtii Dangeard (IAM C-238) described above. We can conclude that the $\delta^{13}C$ content of PR-6 decreases with increasing growth rate, as indicated by the shaded bold line in Fig. 2. In contrast, the low $\Delta \delta^{13}C$ for the data within the circle in Fig. 2 strongly suggests that the effect of CO$_2$ limitation on algal photosynthetic activity lowered the apparent fractionation factor at the rather high growth rates of dense populations within the small incubation tubes. In summary, we conclude that algal $\delta^{13}C$ is governed by two major factors: growth rate and the supply of CO$_2$ as estimated by the above eq. (5).

**Diazotrophs**

Molecular nitrogen has a very strong triple bond between nitrogen atoms. Theoretically, the cleavage of nitrogen atoms under a reversible reaction results in a very large nitrogen isotope fractionation. However, the observed fractionation was small for biological molecular nitrogen fixation, ca. 1.002. Such a small nitrogen isotope fractionation factor strongly suggests that triple-bond cleavage takes place suddenly under irreversible conditions between substrate N$_2$ and an activated substrate. Judging from the present results and from data in the literature, we conclude that isotope fractionation during biological molecular nitrogen fixation is 1.002 in general. This means that the $\delta^{15}N$ supplied via molecular nitrogen fixation is ca. $-2\%$, a fairly low value in marine environments and eutrophic lakes, where the N$_2$-fixing process is easily distinguished by the nitrogen isotope ratio and where N$_2$-fixing cyanobacteria exhibit low $\delta^{15}N$ and high $\delta^{13}C$. These results support the findings of other studies that have demonstrated the significance of molecular nitrogen fixation in the western North Pacific Ocean (Saino & Hattori 1987, Wada & Hattori 1991).

**Epibenthic cyanobacteria from McMurdo Dry Valleys**

The carbon and nitrogen isotope ratios of epibenthic cyanobacteria can be explained by the kinetic isotope effects.

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Table 2. Nitrogen isotope fractionation during cyanobacterial growth in saline ponds of the Labyrinth in the McMurdo Dry Valleys, Antarctica. Nitrogen isotope fractionation factors up to 1.04 were observed during nitrate-uptake processes under high nitrate concentrations.

<table>
<thead>
<tr>
<th>Pond</th>
<th>NH$_4^+$ N (mgN L$^{-1}$)</th>
<th>NO$_3^-$+NH$_4^+$ (mgN L$^{-1}$)</th>
<th>$\delta^{15}$N (NH$_4^+$) (%)</th>
<th>$\delta^{15}$N (NO$_3^-$) (%)</th>
<th>$\delta^{15}$N (cyanobacteria) (%)</th>
<th>$\delta^{13}$C (cyanobacteria) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-11</td>
<td>4.5</td>
<td>455</td>
<td>-22.4</td>
<td>-11.6</td>
<td>-50.0</td>
<td>-4.55</td>
</tr>
<tr>
<td>L-12</td>
<td>10.3</td>
<td>1,370</td>
<td>-21.2</td>
<td>-10.0</td>
<td>-51.4</td>
<td>-6.55</td>
</tr>
<tr>
<td>L-13</td>
<td>6.6</td>
<td>1,290</td>
<td>n.d.</td>
<td>-1.6</td>
<td>-52.7</td>
<td>-7.76</td>
</tr>
<tr>
<td>L-14</td>
<td>8.3</td>
<td>1,370</td>
<td>-8.0</td>
<td>-5.6</td>
<td>-63.4</td>
<td>-13.30</td>
</tr>
<tr>
<td>S-1 Valley</td>
<td>14.2</td>
<td>14.2</td>
<td>+2.0</td>
<td>-13.2</td>
<td>-19.8</td>
<td>-1.50</td>
</tr>
</tbody>
</table>
described above during cyanobacterial growth in McMurdo Dry Valleys. The nitrogen and carbon isotope ratios of microalgae closely depend on the isotope ratios of the substrate used for their growth as well as on their uptake kinetics.

Under substrate-limiting conditions, isotope fractionation factors become close to 1.000 because the diffusion of the substrate into algal cells is the rate-limiting step during the assimilation of inorganic nitrogen, whereas large fractionation factors can occur when enzymatic processes limit the overall reaction under low-light conditions with slow growth rates. As reported by Wada et al. (1984), the δ¹⁵N of sodium nitrate in the McMurdo Dry Valleys is as low as −20‰. Species of cyanobacteria found in the McMurdo Dry Valleys by Matsumoto et al. (1993, 1996) included Lyngbya murrayi, Oscillatoria priestley, and Phormidium priestley. According to Matsumoto, who participated in field surveys in this area from 1976 through 1985, our samples are consistent with these organisms (GI Matsumoto, pers. comm.). The results of our 16S rDNA sequence analysis support the observations of Matsumoto et al. (1993, 1996).

In the present work, we detected two algal groups based on nitrogen isotope ratios, i.e., −20‰ and −80 to −50‰ (Fig. 3). The former group might grow under substrate-limited conditions caused by low nitrate concentrations and/or the aggregation of algal mats at the bottom of saline ponds. However, very low δ¹⁵N values could also occur when algal growth is limited by light intensity under high nitrate concentrations, up to 1,000 mg nitrate-N L⁻¹. In such cases, we expect the occurrence of a high nitrogen isotope fractionation factor (up to ca. 1.04) during nitrate assimilation processes. In McMurdo Dry Valleys, there are several saline ponds with the conditions listed in Table 2.

Relationship between the δ¹³C of marine plankton and sea surface temperature (SST)

The reddish brown Trichodesmium erythraeum (Marumo & Asaoka 1974) has the highest δ¹³C among the pelagic plankton samples documented in the literature (Fig. 4). Nitrogen supply via molecular nitrogen fixation is responsible for the low δ¹⁵N values of Trichodesmium (Wada 1980, Minagawa & Wada 1986). Therefore, Trichodesmium holds a unique position among marine phytoplankton with regard to its carbon and nitrogen isotopic composition (Fig. 4), as described by Carpenter et al. (1997).

The relationship between δ¹³C and growth rate constant was examined, based on all marine phytoplankton δ¹³C data collected from the Pacific Coast off Japan (Fig. 4). Generally, phytoplankton δ¹³C depends on pCO₂, growth rate, SST, nutrient concentration, and the cell geometry of each species (Popp et al. 1998). According to Epplle (1972), seawater temperature and nutrient concentrations are two major factors affecting phytoplankton growth. By accounting for the combined effects of temperature and concentration on nitrate uptake as measured in shipboard experiments, Smith (2010) found evidence of greater temperature sensitivity than that reported by Epplle (1972) for laboratory measurements of growth rate. The lowest δ¹³C values (ca. −30‰) were those of plankton from high-latitude areas (Rau et al. 1982, Goericke & Fry 1994). We could thus expect to see a positive relationship between the growth rate constant and either δ¹³C in phytoplankton (POC) or SST. In fact, we obtained a linear relationship for phytoplankton: δ¹³C=0.25 SST−26.2 (r²=0.59), as indicated in Fig. 4. This linear relationship observed in the western North Pacific off Japan is quite similar to that reported by Goericke and Fry (1994), whose linear regression of δ¹³C_POC on SST was δ¹³C_POC=0.17 [±0.04]×SST−24.6‰ (r=0.72, >5°C in the northern hemisphere).

Therefore, phytoplankton δ¹³C is a possible parameter for deducing in situ growth rate constants in the marine environment. Thus, the variability in δ¹³C content among various types of particulate organic matter in certain ocean waters suggests that stable carbon isotope studies could provide unique information about the growth physiology as well as the carbon dynamics of the plankton community.
An isotopic map of cyanobacteria and plankton

Lake Suwa (36°03′N, 138°05′E) is a typical eutrophic shallow lake in Nagano prefecture, in central Japan, with a surface area of 13.3 km² and a maximum depth of 6.8 m. Blooms of *Microcystis* are often observed from spring to summer in this highly eutrophic lake. *Microcystis* utilizes nitrate from domestic sewage in spring, then uses regenerated ammonium in summer. After that the N₂-fixing cyanobacteria *Anabaena* typically appears under nitrogen-de-
icient and phosphate rich conditions in August. The pattern of succession in this cyanobacterial community provided a useful comparison with the regional differences between marine phytoplankton when plotted on the $\delta^{15}N-\delta^{13}C$ map (Fig. 5a).

Data from a 1985 bloom (Yoshioaka et al. 1994) are shown in Fig. 5b, together with plankton data from the pelagic ocean in Fig. 5c (Wada & Hattori 1991). Furthermore, data from human-impacted lakes in tropical areas in Brazil were also included in Fig. 5a to demonstrate how human activity such as dam construction and sand mining changed $\delta^{15}N$ and $\delta^{13}C$ values of phytoplankton (Wada et al. 1991). Coincidentally, the $\delta^{15}N$ of inorganic nitrogenous compounds in Lake Suwa was quite similar to that of pelagic nitrate (ca. 6%). The isotopic map of pelagic marine phytoplankton, *Microcystis*, and *Anabaena cylindrica*, the $N_2$ fixing cyanobacteria that appear in August in Lake Suwa, Japan, can be divided into three groups (nitrate-rich, nitrate-poor, and $N_2$-fixing communities) depending on nutrient availability, from the nitrate-rich spring season to nitrate-poor-phosphate rich summer season (Fig. 5b).

As shown in Fig. 5a, cyanobacteria and phytoplankton were found in almost the same areas of the isotopic map, strongly suggesting that the isotopic compositions of algae are governed by a growth physiology common among marine algae and cyanobacteria. In the figure, a dramatic increase can be observed in the $\delta^{15}N$ values of phytoplankton in tropical lakes in Brazil affected by human impacts such as sand mining, dam construction, and sewage loading (Wada et al. 1991). Compared to data for marine phytoplankton (the shaded areas in Fig. 5a), the isotopic data for cyanobacteria varied with habitat structure. The lowest value, a $\delta^{15}N$ of $-76.5\%_{oo}$, was obtained from cyanobacteria collected at Lake Bonney, in the McMurdo Dry Valleys (Fig. 3).

**Conclusion**

The relationship between growth rates and carbon isotope fractionation during photosynthesis was investigated using a marine cyanobacterium (*A. quadruplicatum*). Under light-limiting growing conditions, rapid growth of the organism was accompanied by low isotope fractionation factors ($\mu=1.6$, $\alpha=1.017$; $\mu=0.20$, $\alpha=1.024$); that is, $\delta^{13}C$ was positively correlated with the growth rate. Our results also suggest that the $\delta^{13}C$ of marine phytoplankton may be useful for estimating *in situ* growth rates in the open ocean as a first approximation. We also suggest that the isotope of phytoplankton $\delta^{13}C$ and $\delta^{15}N$ over the oceans could help to verify marine ecosystem models by their distributions.

Various marine $N_2$-fixing cyanobacteria were cultured under $N_2$-fixing conditions to elucidate nitrogen isotope fractionation values during $N_2$ fixation. $\delta^{15}N$ ranged from $-2.27$ to $-1.48\%_{oo}$, with an average value of $-1.74\%_{oo}$. The highest $\delta^{13}C$ among pelagic plankton ($-13.6\%_{oo}$) was found for *T. erythraeum*.

The natural abundances of cyanobacteria collected from Antarctic saline ponds in the McMurdo Dry Valleys were measured precisely. Their $\delta^{15}N$ values were divided into two groups, with average values of $-10$ and $-50\%_{oo}$, respectively. $\delta^{13}C$ was highly variable, from $-25$ to $-3\%_{oo}$. The algal growth physiology of these cyanobacteria is discussed on the basis of their carbon and nitrogen isotope ratios.

An isotopic map of cyanobacteria and marine plankton was presented that summarizes data appearing in the present work and in the literature. The isotope ratios of cyanobacteria in natural aquatic ecosystems have extremely wide ranges. Marine phytoplankton and the cyanobacteria of Lake Suwa were divided into three groups (nitrate-rich, nitrate-poor, and $N_2$-fixing communities) based on nutrient availability and growth rate.

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**References**


Goericke R, Fry B (1994) Variations of marine plankton $\delta^{13}$C with latitude, temperature, and dissolved CO$_2$ in the world ocean. Glob Biogeochem Cycl 8: 85–90.


Wada E, Lee JA, Kimura M, Koike I, Reeburgh WS, Tundisi JG,


**Appendix.** Carbon and nitrogen isotope ratios of cyanobacteria collected from the McMurdo Dry Valleys, Antarctica.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample no.</th>
<th>$\delta^{13}$C (%)</th>
<th>$\delta^{15}$N (%)</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Wright Valley</td>
<td>Lake Vanda</td>
<td>V-A-1</td>
<td>−17.5</td>
<td>−10.5 Dried cyanobacteria under a stone</td>
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<tr>
<td></td>
<td>Lake Vanda</td>
<td>V-A-2</td>
<td>−18.5</td>
<td>−10.3 Under sand at 5 cm depth</td>
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<tr>
<td></td>
<td>Lake Vanda</td>
<td>V-A-2*</td>
<td>−17.7</td>
<td>−9.5 Under the ice at 5 cm depth</td>
</tr>
<tr>
<td></td>
<td>Lake Vanda</td>
<td>V-A-3</td>
<td>−18.1</td>
<td>−10.3 Degraded cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Lake Vanda</td>
<td>V-A-5</td>
<td>−7.9</td>
<td>−14.7 Wet cyanobacteria at the edge of the cape at 50 cm depth</td>
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<td>Lake Vanda</td>
<td>V-A-7</td>
<td>−20.7</td>
<td>−12.3 Attached to a rock at the river mouth</td>
</tr>
<tr>
<td></td>
<td>Lake Vanda</td>
<td>X1</td>
<td>−16.0</td>
<td>−17.7 Wet cyanobacteria</td>
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<tr>
<td></td>
<td>Lake Vanda</td>
<td>X4</td>
<td>−15.6</td>
<td>−17.4 Dried cyanobacteria</td>
</tr>
<tr>
<td></td>
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<td>X5</td>
<td>−15.6</td>
<td>−15.9 Dried cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Lake Canopus</td>
<td>C2</td>
<td>−18.5</td>
<td>−4.7 Dried cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Lake Canopus</td>
<td>C3</td>
<td>−9.6</td>
<td>−3.1 Wet cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Lake Canopus</td>
<td>C4</td>
<td>−11.7</td>
<td>−11.8 Wet cyanobacteria</td>
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<tr>
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<td>−14.7</td>
<td>−3.0 Dried cyanobacteria</td>
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<td>Don Juan Pond</td>
<td>D1</td>
<td>−1.8</td>
<td>−19.7 Dried cyanobacteria</td>
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<tr>
<td></td>
<td>Don Juan Pond</td>
<td>D2</td>
<td>−13.0</td>
<td>−19.1 Dried cyanobacteria</td>
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<tr>
<td></td>
<td>Don Juan Pond</td>
<td>D3</td>
<td>−15.1</td>
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<td>Unnamed pond in the Labyrinth</td>
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<td>−5.6</td>
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<td>−17.3 lichen</td>
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<td>L20 lichen B</td>
<td>−25.2</td>
<td>−20.4 Degraded lichen</td>
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<tr>
<td>Taylor Valley</td>
<td>East L. Bonney</td>
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<td>−9.3</td>
<td>−76.5 Wet cyanobacteria near river mouth</td>
</tr>
<tr>
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<td>Melt-stream from Hughes Glacier</td>
<td>B2</td>
<td>−12.7</td>
<td>−10.0 Wet cyanobacteria</td>
</tr>
<tr>
<td></td>
<td>Melt-stream from Hughes Glacier</td>
<td>B3</td>
<td>−12.7</td>
<td>−9.4 Wet cyanobacteria</td>
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