Pergamon

Geochimica et Cosmochimica Acta, Vol. 63, No. 9, pp. 1431–1441, 1999

0016-7037/99 $20.00 + .00

PII S0016-7037(99)00097-6

Nitrogen and carbon isotopic ratios of chlorophyll from marine phytoplankton

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(*Received February* 8, 1999; *accepted in revised form February* 18, 1999)

Abstract—The relationship between the nitrogen and carbon isotopic ratios of chlorophyll *a* and total biomass was explored in cultured marine phytoplankton to assess the utility of chlorophyll as an isotopic proxy for photoautotrophs. A near constant nitrogen isotopic depletion of 5.06 ± 1.13‰ (95% confidence interval) in chlorophyll *a* relative to total nitrogen was observed in 8 species. This value was similar to isotopic differences between chlorophyll *a* and marine particles (5.27 ± 1.48‰ (1σ); $n = 6$) and sediments (5.39 ± 0.67‰ (1σ); $n = 4$) in a variety of settings. These findings suggest that a 5.1‰ isotopic depletion of chlorophyll *a* relative to total algal nitrogen is a robust relationship that justifies the use of chlorophyll as a nitrogen isotopic surrogate for photoautotrophs. Although interspecies differences in $\Delta\delta^{15}N_{cell\text{-}Chla}$ exist, and growth rate has a small effect on this parameter, the field data suggest these factors are probably minimized in the ocean where multiple species and growth rates occur. The nitrogen isotopic depletion of chlorophyll *a* probably occurs during the transamination of glutamic acid in δ-aminolevulinic acid biosynthesis, the first committed precursor to chlorophyll.

The carbon isotopic composition of chlorophyll from 12 batch cultures and 7 species of marine phytoplankton was elevated by 0.32 ± 1.61‰ (95% confidence interval) relative to total cellular carbon. No significant interspecies variance was observed that was not attributable to intraspecies variance. There was a moderate inverse correlation between growth rate and $\Delta\delta^{13}C_{cell\text{-}Chla}$, and it is hypothesized that this parameter is largely responsible for the large range of intraspecies $\Delta\delta^{13}C_{cell\text{-}Chla}$ values observed in batch cultures. *Copyright © 1999 Elsevier Science Ltd*

1. INTRODUCTION

Nitrogen and carbon stable isotopic ratios in marine particles and sediments have been used for the past 4 decades to understand the cycling of the major nutrients between geologic and biologic reservoirs. The timescales of processes studied range between 10^{-2} (e.g., phytoplankton blooms, Altabet et al., 1991) and 10^9 years (e.g., organic carbon burial since the Precambrian, Knoll and Walter, 1992). Often sought with these measurements is the isotopic composition of photoautotrophs that exist at the interface between geologic and biologic domains. However, during heterotrophy and sediment burial, primary isotopic signatures can be significantly altered or obscured (Benner et al., 1991; DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Jasper and Hayes, 1990; Montoya, 1994; Sachs, 1997; Sachs and Repeta, subm; Wada, 1980).

For instance, due to the admixture of terrestrial carbon with autocthonous algal material, a reconstruction of atmospheric carbon dioxide partial pressures from sedimentary $\delta^{13}C$ in the Gulf of Mexico yielded incongruent values up to 3 times greater than those unambiguously determined from bubbles trapped in a polar ice core (Jasper and Hayes, 1990). The carbon isotopic analysis of known algal material (Jasper and Hayes, 1990) from that same site yielded *p*CO2 values in agreement with the ice core record. Likewise, a historical record of sedimentary $\delta^{15}N$ (Calvert et al., 1992) in the Eastern Mediterranean Sea was initially interpreted as evidence for increased nutrient availability during the repeated deposition of Late Pleistocene sapropels in that basin. Analyses of algal chlorophyll $\delta^{15}N$ demonstrated that the bulk sediment isotopic signal was a result of diagenesis (Sachs, 1997; Sachs and Repeta, subm.).

The necessity therefore exists to perform nitrogen and carbon isotopic measurements on biomarkers, or molecular fossils having a definitive biological (i.e., algal) origin (Hayes et al., 1990; Hayes et al., 1989; Hayes et al., 1987). Although significant progress has been made on this front for carbon, few studies have developed protocols for the nitrogen isotopic analysis of biomarkers. An ideal substance for compound-specific $\delta^{15}N$ studies is chlorophyll. Chlorophyll is a photosynthetic pigment found in all photoautotrophs. Its nitrogen-containing macrocycle is resilient and can be recovered from marine particles and sediments (Louda and Baker, 1986). Transformation reactions during cell senescence (Hendry et al., 1987; Spooner et al., 1994), grazing (Downs, 1989; Head, 1992; Shuman and Lorenzen, 1975) and microbial activity (Sun et al., 1991; Sun et al., 1993) can alter the functional groups attached to the chlorophyll macrocycle, leaving the nitrogen-containing moieties intact. Thus, chlorophyll degradation products such as the pheophytins and pheophorbides (Harris et al., 1995) which are more abundant than chlorophyll in sediments, can substitute for intact chlorophyll in biomarker $\delta^{15}N$ studies (Sachs, 1997).

The purpose of this study was to determine the utility of chlorophyll as a nitrogen and carbon isotopic surrogate for marine phytoplankton. In particular, we undertook this study in order to demonstrate that a predictable relationship existed between chlorophyll and total cellular $\delta^{15}N$ and $\delta^{13}C$ values in phytoplankton. A few published reports have indicated a linear correspondence between chlorophyll and plant δ values, primarily

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Table 1. Description of cultures. All were grown in f/2 medium with the exception of *Synechococcus* sp., which was grown in SN2 medium. CCMP is an abbreviation for the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (McKown Point, West Boothbay Harbor, ME 04575). Full names of species can be found in Table 2. J. Montoya (Harvard University) and J. Waterbury (Woods Hole Oceanographic Institution) supplied the seed cultures denoted JM and JW, respectively. The temperature "Rm T" refers to cultures that were maintained at room temperature, which fluctuates between ca. 20 and 24°C.

Clone	Abbrev. name	Origin	Start Date	End Date	Culture size	Total Chla (mg)	L:D Cycle	T (°C)
TW	TW1	JM	6/22/94	7/5/94	20 L	5.58	12:12	18
CCMP1336	TW4	CCMP	11/1/95	11/8/95	2 × 1.5 L	2.24	24:0	Rm T
T-Iso	IG1	JM	6/22/94	7/5/94	20 L	7.36	12:12	18
CCMP1323	IG3	CCMP	7/26/95	8/8/95	2 × 1 L	0.76	24:0	Rm T
CCMP1320	DUN2	CCMP	5/11/95	5/17/95	20 L	6.34	12:12	18
CCMP1320	DUN4	CCMP	11/1/95	11/8/95	3 × 1 L	4.09	24:0	Rm T
CCMP1314	AMP2	CCMP	5/15/95	5/26/95	20 L	1.43	12:12	18
CCMP1314	AMP4	CCMP	11/1/95	11/10/95	2 × 1.5 L	3.37	24:0	Rm T
CCMP630	PHA2	CCMP	5/11/95	5/16/95	20 L	13.85	12:12	18
CCMP630	PHA4	CCMP	11/1/95	11/8/95	3 × 1 L	3.12	24:0	Rm T
CCMP1325	PAV2	CCMP	5/11/95	5/17/95	20 L	8.67	12:12	18
CCMP1325	PAV4	CCMP	11/1/95	11/9/95	2 × 1.5 L	1.27	24:0	Rm T
WH7803	SYN2	JW	5/22/95	6/6/95	3 × 1 L	1.12	12:12	18
CCMP373	EH5	CCMP	10/11/97	11/3/97	20 L	24.78	14:10	23

in terrestrial plants. The single investigation of this relationship for nitrogen was performed on 6 terrestrial plant and grass specimens and 1 phytoplankton species, and found a non-unity slope of 1.3 and a near-zero intercept in a plot of $\delta^{15}N_{plant}$ versus $\delta^{15}N_{Chla}$ [$\delta^{15}N_{plant} = (1.30)\delta^{15}N_{Chla} - 0.40$, $r^2 = 0.90$ (Kennicutt II et al., 1992)]. More work has been done for carbon, but of the 19 analyses, only 2 were for phytoplankton. A plot of $\delta^{13}C_{plant}$ versus $\delta^{13}C_{Chla}$ had a near-unity slope and an intercept of 2.74 [$\delta_{13}C_{plant} = (1.09)\delta^{13}C_{Chla} + 2.74$, $r^2 = 0.91$ (Laws et al., 1995; and references therein)]. There is no indication from these studies that similar relationships are valid for marine phytoplankton. Therefore, we present results from experiments with algal cultures that establish chlorophyll *a* as a nitrogen and carbon isotopic proxy for phytoplankton.

2. EXPERIMENTAL METHODS

2.1. Culturing Procedures

Axenic cultures of marine algae were grown using f/2 medium (Guillard, 1975) or SN2 medium (*Synechococcus* sp. only; Waterbury et al., 1986) in 2.5 L low form culture flasks, 2.8 L Fernbach flasks and 20 L carboys (Table 1). Cultures were maintained at 18°C (23° for EH5) or room temperature at constant light or a 12-hour (14:10 for EH5) light-dark cycle (Table 1; average irradiance = 450 $\mu E/m^2/sec$; 4 × 25 W × 48″ Ace Cool White Shop Lights) and were aerated by swirling twice daily (2.5 and 2.8 L flasks) or bubbling with 0.2 μm-filtered laboratory air (20 L carboys). Cultures were harvested at the late stage of exponential growth as determined by in situ fluorescence or absorption (Table 1). Growth rates were determined from the exponential part of the growth curve, such that the coefficient, μ, in an exponential fit to the fluorescence or absorbance data (e.g., Flu or Abs = $ae^{\mu t}$) yielded rates in units of day^{-1}.

2.2. Chlorophyll Extraction and Purification of Pheophytin

Cultures were harvested by vacuum-filtration through a pre-combusted (450°C, >8 h) 293 mm Gelman A/E filter. In all but 2 instances (in which filters were stored at −20°C), filters were immediately stored in liquid nitrogen. Just prior to extraction, filters were thawed at room temperature and two 1 cm-diameter subsamples were removed with a cork borer for whole-cell $\delta^{15}N$ and $\delta^{13}C$ analyses. Filters were ultrasonically extracted (3×) in 125 mL degassed acetone containing 5 g $NaHCO_3$. The extracts were filtered through a Whatman 47 mm GF/F filter, and the filtrate was sparged with N_2 during subsequent extractions. Combined extracts were poured into a 2 L separatory funnel containing 125 mL water, and the chlorophyll was partitioned (3×) into 200 mL hexane. Chlorophyll in both the combined hexane fractions and the aqueous fraction was quantified spectrophotometrically at 664 nm in methylene chloride. Typically, no chlorophyll *a* remained in the aqueous fraction. The combined hexane fractions were then back-extracted (1×) with 200 mL 15/85 H_2O/MeOH to remove carotenoids, and the chlorophyll was re-quantified as above.

Chlorophyll demetallation (to form pheophytin) was achieved by adding 200 mL 10% HCl (aq) to the hexane fraction and shaking for 1 min. The aqueous fraction was poured off and the hexane neutralized with 100 mL 2% (w/v) $NaHCO_3$ (aq), dried over Na_2SO_4, and rotary-evaporated to dryness. This procedure is not expected to cause isotopic fractionation since the demetallation reaction goes to completion, as evidenced by the pure pheopigment visible absorbance spectrum. The sample was redissolved in 1 mL methylene chloride, spectrophotometrically quantified at 666 nm, then dried and stored under nitrogen at −20°C.

Pheophytin *a* was purified using reversed (C_{18}) - and normal-phase (SiO_2) HPLC. Dried extracts were re-dissolved in a small volume (100–500 μL) of $MeCl_2$. Between two and five 50 to 200 μL injections (containing 1 to 25 mg of extract or 0.5 to 4 μmol pheophytin *a*) were made onto a Waters 660 HPLC with photodiode array (Waters 990, Milford, MA) or fluorescence (Hitachi F-1000, Tokyo, Japan) detectors fitted with a 10 mm I.D. × 250 mm preparative C_{18} column (5 μm particle size; Kromasil Kr100-5-C18 Eka Nobel, Bohus, Sweden) to which was attached a 10 mm I.D. × 50 mm C_{18} guard column (Kromasil). An acetone (solvent A) and methanol (solvent B) gradient was employed as follows: (time-min, flow-mL/min, %A) : (0, 6, 95), (10, 6, 70), (15, 7, 65), (30, 6, 0). Pheophytin *a*, eluting at ca. 20 min, was separated from its allomer, pheophytin *a′* peaks, eluting at about 21 min, and collected into an 18 mL glass vial with a Gilson fraction collector. The pheophytin *a* fraction was then rotary-evaporated to dryness and stored at −20°C. Between samples the column was cleaned with 90 mL 100% $MeCl_2$.

Chlorin fractions were redissolved in 100–400 μL of 10% acetone in hexane prior to SiO_2 HPLC. Two to four injections of 50 to 200 μL (corresponding to 0.5 to 2 mg pheophytin *a*) were made onto a 4.6 mm I.D. × 100 mm Alltech Spherisorb silica analytical column with 3 μm particle size (Alltech Associates, Inc, Deerfield, IL). Isocratic elution with 4% acetone in hexane at 2 mL/min separated pheophytin *a′*, eluting at ca. 11 min, from pheophytin *a*, eluting at ca. 13 min. Pheophytin *a* was collected into a 4 mL glass vial with a fraction collector, dried under a N_2 stream, and stored at −20°C. Care was taken to collect the entire pheophytin *a* peak during chromatography in order to prevent isotopic alteration of a sample resulting from across-peak isotopic variations (Bidigare et al., 1991). Between samples the

column was cleaned with 60 mL 100% acetone and re-equilibrated with 4/96 acetone/hexane for 30 min.

Spectrophotometrically-determined pheophytin *a* recoveries for the entire purification procedure were 88% (±18%), or about 90–95% for each step. Nitrogen purity (i.e., the total nitrogen in the sample attributable to pheophytin *a*), determined by elemental analysis on 6 of the cultures, averaged 95.9 ± 3.4%. Carbon purity averaged 91.2 ± 4.3%. Two samples, prepared in tandem, could be processed in about 8 h.

2.3. Isotope Analyses

Purified pheophytin *a* was transferred to smooth-walled tin capsules (8 × 6 mm; Elemental Microanalysis, Manchester, MA) in 200 μL of acetone and dried under a 60 W light bulb. Filter sub-samples for whole-cell isotopic analysis were dried at 60°C and placed into tin boats (5 × 9 mm; Elemental Microanalysis). Tin capsules and boats were folded with forceps and stored in a desiccator until isotopic analysis. Nitrogen and carbon isotope ratios were measured on a Finnigan MAT delta S isotope ratio mass spectrometer connected to a CHN analyzer (Heraeus Rapid Elemental Analyzer) by an automated "trapping box" for the sequential cryogenic purification of CO_2 and N_2 (Stable isotope Laboratory at the Marine Biological Laboratory, Woods Hole, MA, Fry et al., 1992), thus permitting rapid $\delta^{15}N$ and $\delta^{13}C$ analyses on the same sample. Standard delta notation is used for reporting stable isotopic ratios of nitrogen and carbon:

$$\delta^{n}X \equiv \left[\frac{({}^{n}X/{}^{(n-1)}X)_{smpl.}}{({}^{n}X/{}^{(n-1)}X)_{std.}} - 1\right] \times 1000‰ \quad (1)$$

where ${}^{n}X = {}^{15}N$ or ${}^{13}C$, and the isotopic standards for C and N are Peedee Belemnite limestone ($\delta^{13}C \equiv 0‰$; Craig, 1953) and atmospheric N_2 ($\delta^{15}N \equiv 0‰$, (Hoering, 1955). Differences of delta values are reported as $\Delta\delta^{n}X_{cell\text{-}Chla}$.

The measurement precision for $\delta^{15}N$ and $\delta^{13}C$ determinations in this study was 0.31 and 0.38‰, respectively, the mean spread for 48 ($\delta^{15}N$) and 30 ($\delta^{13}C$) duplicate analyses of chlorin and POM samples. The precision of $\Delta\delta$ determinations is better than 0.74 and 2.00‰, respectively, for nitrogen and carbon, the root mean square error, or average dispersion of n cultures for each species around the species mean (Sokal and Rohlf, 1981):

$$= \sqrt{\frac{\sum_{i=1}^{a}\sum_{i\text{-}1}^{n} Y_i^2 - \sum_{i=1}^{a}\frac{\left(\sum_{i=1}^{n} Y_i\right)^2}{n_i}}{\sum_{i=1}^{a} n_i - a}}$$

where Y is the measured $\Delta\delta$ value of the a′th species measured n times. This statistic thus describes all variance in the analysis and intraspecies physiochemistry, including growth rate effects.

Throughout the remainder of the manuscript we use the term "chlorophyll" when referring to purified pheophytin *a* from our phytoplankton cultures. This approach is justified since the demetallation reaction of chlorophyll *a* to form pheophytin *a* was taken to completion.

3. RESULTS

The mean nitrogen isotopic depletion of chlorophyll *a*, relative to total cellular nitrogen, in all cultures was 5.08 ± 0.87‰ (95% confidence interval (CI); $n = 19$). Chlorophyll carbon was enriched in ^{13}C by 0.52 ± 2.35‰ (95% CI; $n = 13$; Table 2) relative to total carbon. Box plots (Fig. 1) reveal 2 outliers—samples that fall beyond 1.5 times the interquartile range (IQR) of the data (Moore and McCabe, 1993). *Synechococcus* sp. had $\Delta\delta^{15}N_{cell\text{-}Chla} = 10.13‰$ (fig. 1a) and one culture of *Amphidinium carterae* had $\Delta\delta^{13}C_{cell\text{-}Chla} = 10.58‰$ (Fig 1c). When the 2 outliers are omitted from the averages the mean cell-chlorophyll isotopic differences are 4.80 ± 0.68‰ for nitrogen (95% CI; $n = 18$) and −0.32 ± 1.61‰ for carbon (95% CI; $n = 12$).

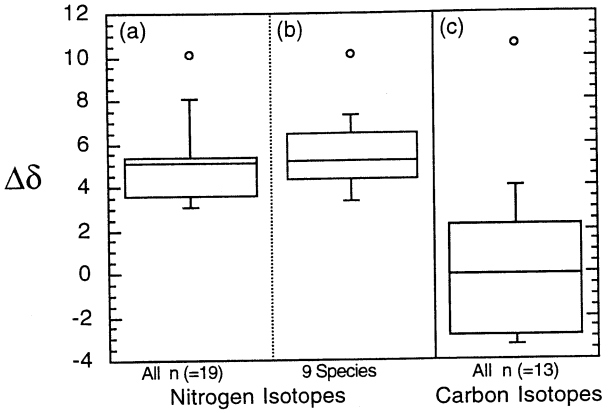


Fig. 1. Box plots of the isotopic difference between chlorophyll and whole cells constructed to identify potential outliers in the data. The $\Delta\delta^{15}N_{cell\text{-}Chla}$ for 19 individual cultures were used to construct box plot (a), while the average $\Delta\delta^{15}N_{cell\text{-}Chla}$ for each of 9 species groupings was used to construct (b). *Synechococcus* sp. is an outlier both when the data are analyzed together and in species groupings. All 13 $\Delta\delta^{13}C_{cell\text{-}Chla}$ determinations were used to construct box plot (c), showing *Amphidinium carterae* to be an outlier. Since no significant interspecies $\Delta\delta^{13}C_{cell\text{-}Chla}$ differences were observed, an outlier test was not performed on species groupings.

An analysis of variance (ANOVA) was performed to determine whether variance between species existed that was not attributable to variance between cultures from the same species (Sokal and Rohlf, 1981). A significant ($P < 0.01$; $n = 8$) added variance component between phytoplankton species for cell-chlorophyll nitrogen isotopic differences was found. A recalculation of the average cell-chlorophyll nitrogen isotopic

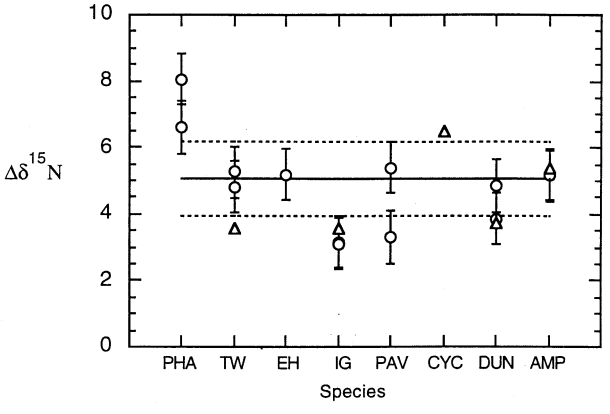


Fig. 2. The nitrogen isotopic difference between chlorophyll *a* and whole cells in 8 species of marine phytoplankton, where $\Delta\delta^{15}N = \Delta\delta^{15}N_{cell\text{-}Chla} = \delta^{15}N_{cell} - \delta^{15}N_{Chla}$. Plotted values are in per mil (‰). Phytoplankton species abbreviations are listed in Table 2. Open circles correspond to cultures prepared for this study. Open triangles correspond to cultures previously prepared by R. Goericke and J. Montoya (unpubl. data). The error bar is the root mean square error for the culture experiments, 0.74‰, and represents a lower limit on the precision of the $\Delta\delta^{15}N_{cell\text{-}Chla}$ analyses. Also plotted (solid line) is the average chlorophyll-cell isotopic difference for all 8 species, 5.06‰, and the 95% confidence interval for that value, ±1.13‰ (dotted lines).

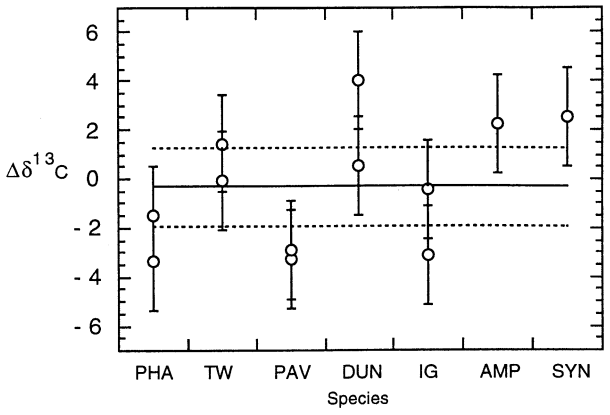


Fig. 3. The carbon isotopic difference between chlorophyll *a* and whole cells in seven species of marine phytoplankton. The abbreviations represent the species listed in Table 1. Duplicate cultures of each species, except AMP and SYN, were prepared and the $\Delta\delta^{13}C_{cell\text{-}Chla}$ for each experiment is plotted, with a line connecting the 2 results. Also plotted (solid line) is the average chlorophyll-cell isotopic difference for the 12 cultures, −0.32‰, and the 95% confidence interval for that value, ±1.61‰ (dotted lines).

difference by species grouping, as dictated by the ANOVA results, yields 5.06 ± 1.13‰ (95% CI; $n = 8$; Fig. 2). When the outlier test is performed on species groupings *Synechococcus* sp. ($\Delta\delta^{15}N_{cell\text{-}Chla} = 10.13‰$) remains an outlier: ($\Delta\delta^{15}N_{cell\text{-}Chla} = 10.13‰$) > (1.5 × IQR = 9.65‰; Fig. 1b). A likely explanation for this result is the infection of the SYN2 culture by heterotrophic bacteria.

Nitrogen isotopic values increase by ca. 3.5‰ per trophic step (DeNiro and Epstein, 1981; Altabet, 1988). Since whole cell $\delta^{15}N$ values were determined on total particulate matter (>1μm nominal pore size), this value may have been elevated by the presence of microheterotrophs in the culture. Evidence supporting this possibility is the elevated $\delta^{15}N$ value of PON (3.9‰) relative to nitrate (2.0‰) in the SYN2 culture medium. Phytoplankton are typically depleted in ^{15}N relative to DIN when nutrients are abundant (Altabet and Francois, 1994), the result of a kinetic isotope effect (Wada and Hattori, 1978). Since nitrate concentrations in the medium were high (880 μM), all cultures (except SYN2) had PON $\delta^{15}N$ values lower than nitrate by −0.5 to −9‰ (average = −4.2 ± 2.9‰, $n = 12$, Sachs, 1997). In contrast, the PON in the SYN2 culture was +1.9‰ enriched in ^{15}N compared to nitrate. We therefore suspect that the high $\Delta\delta^{15}N_{cell\text{-}Chla}$ value obtained for the *Synechococcus* culture resulted from the presence of heterotrophic bacteria having a higher $\delta^{15}N$ than the cyanobacterium.

The results of ANOVA for carbon indicate no significant ($P > 0.05$; $n = 6$) interspecies variance in the cell-chlorophyll carbon isotopic difference (Fig. 3), irrespective of the inclusion of the AMP4 culture. Since all variance in measured $\Delta\delta^{13}C_{cell\text{-}Chla}$ can be explained by within-species variability, AMP4 remains an outlier (Fig. 1c), and the cell-chlorophyll carbon isotopic difference remains −0.32 ± 1.61‰ (95% CI; $n = 12$).

4. DISCUSSION

Chlorophyll *a* is markedly depleted in ^{15}N (Fig. 2) and slightly enriched in ^{13}C (Fig. 3) relative to total algal nitrogen and carbon. Published $\Delta\delta^{13}C_{plant\text{-}Chla}$ values from 2 prior studies of different plants and algae span a similar range of values to those we found (Table 2; Galimov and Shirinsky, 1975; Kennicutt II et al., 1992). However, a lesser nitrogen isotopic depletion of 1.71 ± 2.44‰ (95% CI) was reported in an investigation of terrestrial plants (Table 2; (Kennicutt II et al., 1992). The marine macroalga, *Fucus* sp., that we studied from Vineyard Sound, MA had a chlorophyll *a* isotopic depletion of 2.6‰, relative to the whole plant (Table 2), reinforcing the likelihood that chlorophyll *a* in higher plants is less isotopically depleted relative to whole plant nitrogen than in phytoplankton. Below we discuss the origin and inter-species variation in chlorophyll *a* isotopic depletion relative to total algal nitrogen.

4.1. Isotopic Fractionation during Chlorophyll Biosynthesis

There are 5 instances in the synthetic pathway of chlorophyll *a* where bonds to nitrogen are formed or broken, and hence, where N isotopic fractionation might be expected to occur. The first 2 are, respectively, the transaminations of α-ketoglutaric acid (AKA; Fig. 4) and glutamate-1-semialdehyde in the formation of glutamic acid (GLU; Fig. 4) and δ-aminolevulinic acid (ALA; Fig. 4)—the first committed precursor to chlorophyll in all plants (Beale and Weinstein, 1991). The condensation of 2 ALAs to form porphobilinogen (PBG; Fig. 4) and the deamination of 4 PBGs to form hydroxymethylbilane (Fig. 4) are the third and fourth. And the metal insertion reaction of protoporphyrin 9 to yield Mg protoporphyrin IX is the fifth instance where N isotopic fractionation may be imparted to chlorophyll. The reaction sequence and all enzymes in the transformation of glutamate into chlorophyll *a* are the same in all plants and algae (Beale and Weinstein, 1991; Leeper, 1991).

Of the 5 enzyme-catalyzed reactions only transamination has been studied for N isotopic effects (Macko et al., 1986). Large isotope effects were observed for the reaction of GLU and oxaloacetic acid (OAA) to form AKA and aspartic acid (ASP) by the enzyme porcine-heart glutamic oxaloacetic transaminase. ASP thus produced was depleted in ^{15}N by 8.3‰, relative to the GLU from which it was synthesized. The transamination of glutamic acid to form ALA is the first dedicated reaction in chlorophyll biosynthesis (Beale and Weinstein, 1991) making it a likely contributor to the isotopic depletion of chlorophyll. In addition, since GLU is the precursor to most cellular nitrogenous species (Zubay, 1983) it may accumulate in parts of the cell. This is a prerequisite for isotopic fractionation of a biosynthetic product since the complete conversion of a substrate to a product in a closed system results in zero net isotopic fractionation (Mariotti et al., 1981).

The third instance where N isotopic fractionation may be imparted to chlorophyll is in the condensation of 2 ALAs, catalyzed by the enzyme δ-aminolevulinate dehydrase, to form PBG (Fig. 4). Isotope effects can be expressed in this reaction even if 100% of ALA is converted to PBG since there are 2 identical amine groups available for reaction. If δ-aminolevulinate dehydrase posseses an isotope effect then the pyrrole

Table 2. Compilation of nitrogen and carbon isotopic measurements in chlorophyll from plants and algae from this study and the literature. All isotope values from this study are averages of 2 to 4 measurements except for cultures IG3 and PAV4 (for which single isotopic analyses were made for C and N on both chlorophyll and whole cells), and TW4 (for which single isotopic analyses were made for C and N on whole cells). All chlorophyll isotopic values were performed on pheophytin *a*, the de-metalllated form of chlorophyll *a*, except for the data from Kennicutt et al. (1992), which average isotope values for chlorophylls *a* and *b*.

				Nitrogen isotopes			Carbon isotopes		
Species	Plant type	Sample	μ	Cell	Chl *a*	$\Delta\delta^{15}N_{cell\text{-}Chla}$	Cell	Chl *a*	$\Delta\delta^{13}C_{cell\text{-}Chla}$
Thalassiosira weissflogii	Mar. Phyt	TW1		−2.20	−7.00	4.80	−8.25	−8.20	−0.05
	Mar. Phyt	TW4		−7.30	−12.55	5.25	−11.60	−13.05	1.45
Isochrysis galbana	Mar. Phyt	IG1		0.55	−2.60	3.15	−10.75	−10.30	−0.45
	Mar. Phyt	IG3		1.30	−1.80	3.10	−14.00	−10.90	−3.10
Dunaliella tertiolecta	Mar. Phyt	DUN2	0.47	1.65	−3.20	4.85	−24.10	−24.60	0.50
	Mar. Phyt	DUN4	0.34	0.30	−3.55	3.85	−16.20	−20.20	4.00
Amphidinium carterae	Mar. Phyt	AMP2	0.34	−1.55	-6.70	5.15	−24.75	−27.00	2.25
	Mar. Phyt	AMP4	0.54	−0.75	−5.93	5.18	−3.55	−14.13	10.58
Phaeodactylum tricornutum	Mar. Phyt	PHA2	1.1	−3.10	−11.15	8.05	−9.15	−5.80	−3.35
	Mar. Phyt	PHA4		1.85	−4.75	6.60	−9.25	−7.75	−1.50
Pavlova lutheri	Mar. Phyt	PAV2	0.56	−4.10	−7.40	3.30	−16.20	−12.95	−3.25
	Mar. Phyt	PAV4	0.7	−0.10	−5.50	5.40	−14.50	−11.60	−2.90
Emiliania huxleyi	Mar. Phyt	EH5		−1.10	−6.28	5.18			
Synechococcus sp.	Mar. Phyt	SYN2		3.88	−6.25	10.13	−15.65	−18.20	2.55
Fucus sp.	Mar. Macroalg.	Fucus		6.96	4.33	2.63			
R. Goericke and J. Montoya (unpubl. data)									
Thalassiosira weissflogii	Mar. Phyt			−4.48	−7.86	3.38			
Isochrysis galbana	Mar. Phyt			−2.04	−5.63	3.59			
Dunaliella tertiolecta	Mar. Phyt			0.95	−2.78	3.73			
Amphidinium carterae	Mar. Phyt			0.79	−4.59	5.38			
Cyclotella fusifornis	Mar. Phyt			1.02	−5.45	6.47			
Kennicutt et al. (1992)									
Ambrosia artomisifolia	Terr. Plant			7.90	7.40	0.50	−30.10	−25.35	−4.75
Petroselinum crispum	Terr. Plant			4.00	4.85	−0.85	−27.70	−27.25	−0.45
Brassica gemmifera	Terr. Plant			8.60	8.00	0.60	−25.00	−24.65	−0.35
Sorghum halepense	Terr. Plant			6.40	5.05	1.35	−16.30	−15.30	−1.00
Cynodon dactylon	Terr. Plant			19.90	15.60	4.30	−16.50	−15.55	−0.95
Sorghum bocolor	Terr. Plant			13.80	7.45	6.35	−18.30	−15.85	−2.45
Isochrysis	Phyt.			−1.50	−1.20	−0.30	−13.50	−12.20	−1.30
Galimov and Shirinsky (1975)									
Anabaena variabilis	Freshwtr. Phyt						−27.30	−28.40	1.10
Lupinus luteus L.	Terr. Plant						−27.60	−29.50	1.90

formed should be depleted in ^{15}N, and the ^{15}N-enriched terminal amine group would be lost in the subsequent reaction. In a similar reaction, the dehydration of GLU by glutamate dehydrogenase was shown to possess a large nitrogen isotope effect (Schimerlik et al., 1975).

The next step in chlorophyll biosynthesis, the condensation and deamination of 4 PBGs, by the enzymes PBG deaminase and uroporphyrinogen cosynthetase (Leeper, 1991), is not expected to result in significant isotopic fractionation since bonds to pyrrolic nitrogen are neither formed nor broken. Additionally, it is assumed that tight feedbacks exist to prevent the accumulation of products not lying at biosynthetic branch points, and that the energy expenditure for the synthesis of such products is large, making their subsequent destruction unlikely. Radiolabeled pigment turnover experiments support these suppositions (Goericke and Welschmeyer, 1992).

The final potential source of N isotopic fractionation during chlorophyll biosynthesis occurs during the metallation of protoporphyrin IX (PTP), by Mg-chelatase, to form Mg-protoporphyrin IX. PTP lies at a branch point in tetrapyrrole synthesis within the chloroplast (Beale and Weinstein, 1991), where metallation with either Mg or Fe occurs in the production of chlorophylls (Mg) and hemes (Fe). Thus the incomplete metallation of PTP with Mg for chlorophyll synthesis, and a switch to heme production, could impart ^{15}N depletion to chlorophyll if Mg-chelatase has an isotope effect. That enzyme has not been studied for isotope effects, but equilibrium metal exchange reactions between Mg-meso-tetraphenylporphin and its demetallated form indicated significant (i.e., 2.2‰) isotopic depletion in the metallated compound (Macko, 1981). The observations that Mg-porphyrin intermediates do not typically accumulate in the chloroplast, and that heme and chlorophyll production appear to be separated in time (the former occurring in the dark, the latter in the light; Beale and Weinstein, 1991), suggest that this biosynthetic step is not a significant source of nitrogen isotopic depletion of chlorophyll.

4.3. Interspecies variation in chlorophyll ^{15}N-depletion

Small but significant ($P < 0.01$; $n = 8$) differences in $\Delta\delta^{15}N_{cell\text{-}Chla}$ between phytoplankton species exist that may result from variations in the fraction of nitrogen contained in non-protein biochemicals. Most of the nitrogen in marine phytoplankton is contained in proteins, with smaller fractions in

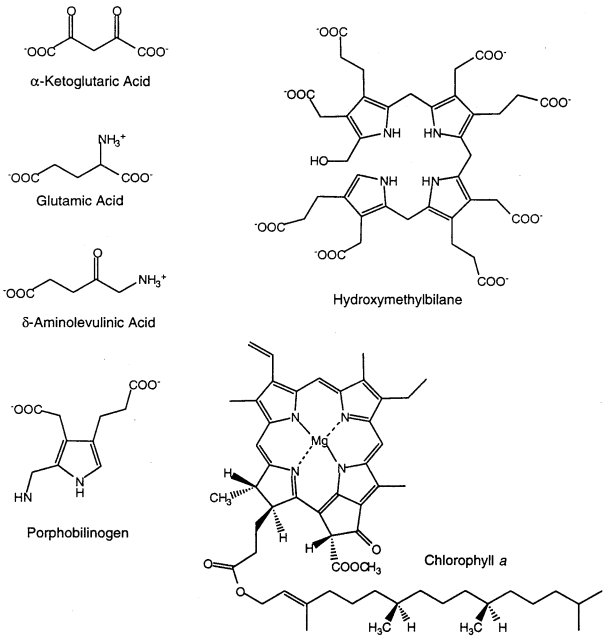


Fig. 4. The structures of key intermediates in the biosynthesis of chlorophyll *a*. Adapted from Zubay (1983) and Beale and Weinstein (1991).

chlorophyll, nucleic acids and amino sugars. Assuming a Redfield C/N ratio of 6.6, and a carbon/chlorophyll (w/w) ratio of 30 (Parsons et al., 1984), the following cellular weight percentages of N are calculated: amino acids, 86.8% (±4.6%); nucleic acids, 1.5 to 10.5%; and chlorophyll, 1.2%, leaving 1.5 to 10.5% of cellular nitrogen remaining in all other minor components such as amino sugars. Some diatoms have been found to contain significant quantities of chitin, a polymer of the amino sugar *N*-acetyl-D-glucosamine, such that amino sugar nitrogen may amount to 15–20% of total cellular nitrogen in some instances (Smucker and Dawson, 1986). Glutamic acid (GLU) is the precursor to this array of nitrogenous biochemicals (Zubay, 1983).

A simple conceptual model is therefore proposed in which isotopic differences between cellular nitrogenous species stem from a hierarchy in the transfer of GLU to the biosynthetic pathways of minor components and protein. The mass balances utilize the few published nitrogen isotopic data available for individual biochemicals in plants, and in certain instances, animals. The isotopic differences for plants may vary substantially. Briefly, protein was enriched in ^{15}N by 3.53 (±0.29; 1σ)‰, relative to whole cells, in 6 species of algae and macroalgae (Macko et al., 1987). Chlorophyll was depleted in ^{15}N by 5.06 (±1.13; 95% CI)‰ in 8 species of marine phytoplankton (this study). The amino sugar, N-acetylglucosamine, which occurs in diatoms and other phytoplankton (Smucker and Dawson, 1986), was depleted in ^{15}N by about 9‰ relative to whole arthropods (Schimmelman and DeNiro, 1986). Insect chitin was also depleted in ^{15}N relative to whole organisms (Deniro and Epstein, 1981), as were zooplankton molts (Montoya et al., 1992); the latter by 3.8 to 4.6‰. Finally, nucleic acids were reported to be depleted in ^{15}N relative to whole algal cells by about 2‰ (Dr. Luis Cifuentes, personal communication).

From mass balance considerations non-protein nitrogen must be depleted in ^{15}N. That is, $\delta^{15}N_{cell} = (3.5)\ (0.87) + \delta^{15}N_{non\text{-}protein}\ (0.13)$, resulting in $\delta^{15}N_{non\text{-}protein} = -23.4‰$ when $\delta^{15}N_{cell}$ is normalized to zero. The ^{15}N-depletion in non-specified cellular nitrogen (1.5 to 10.5% of total N) is even greater when experimentally-determined isotopic values for chlorophyll, nucleic acids and amino sugars are included in the mass balance. Assuming nucleic acids plus amino sugars sum to 5% of total cellular N and have a mean isotopic value of −10‰ leaves 7% of celluar N in non-specified biochemicals having an isotopic depletion of −35.6‰. That is, $\delta^{15}N_{cell} = (3.5)\ (0.87) + (-5)\ (0.01) + (-10)\ (0.05) + \delta^{15}N_{other}\ (0.07)$, and $\delta^{15}N_{other} = -35.6‰$ when $\delta^{15}N_{cell} = 0$. Increasing the $\delta^{15}N$ of nucleic acids plus amino sugars or decreasing the contibution of non-specified N below 7% exacerbates the isotopic depletion of this material.

The large isotopic depletions required for non-protein cellular nitrogen strongly suggest that published protein $\delta^{15}N$ data are not representative of average marine phytoplankton. An alternative estimate of protein $\delta^{15}N$ can be calculated by assuming that non-protein N sums to 13% of total cellular nitrogen (i.e., 100 − 87 = 13%) and has an isotopic value of −5‰. This value is plausible, since all non-protein materials measured to date are depleted in ^{15}N by 2 to 9‰ relative to total nitrogen. Furthermore, the mass balance simplifies to the 2 values we are most confident in; namely the isotopic value of chlorophyll (this study), and the percent of algal nitrogen contained in protein (Parsons et al., 1984). Protein is thus calculated to be enriched in ^{15}N by 0.75‰, relative to total cellular nitrogen.

We propose that enzyme-catalyzed transaminations of glutamic acid to form the precursors of chlorophyll, nucleic acids and amino sugars exhibit kinetic isotope effects that result in ^{15}N-depletions of these products, and compensatory isotopic enrichments of the substrate (glutamic acid). As mentioned, enzymatic transaminations have been demonstrated experimentally to result in a significant isotopic depletion of the product (Macko et al., 1986). The branch points for non-protein biosynthetic pathways must then occur prior to that for protein synthesis. This would provide a mechanism for enriching a pool of glutamic acid in ^{15}N as transamination reactions removed the amino acid for the synthesis of non-protein nitrogenous species, resulting in an isotopic enrichment of protein.

The mass balance model can be used to explain the interspecies variation in the chlorophyll-cell nitrogen isotopic difference we observed in cultured phytoplankton. Using the observed variation in the fraction of algal nitrogen contained in protein, 86.8 ± 4.6% (Parsons et al., 1984), and assigning protein a $\delta^{15}N$ of 0.75‰, results in a range of −3.5 to −8.0‰ for the isotopic composition of non-protein nitrogen (fig. 5). In these calculations we assumed that protein $\delta^{15}N$ is invariant between algal species because Macko, et al (1987) reported less than 10% variation in $\Delta\delta^{15}N_{cell\text{-}protein}$ amongst 6 species of cultured phytoplankton. If that assumption is valid, the relatively small range of interspecies $\Delta\delta^{15}N_{cell\text{-}Chla}$ values we observed in algal cultures (figs. 2 and 5) can be explained by differencess in the fraction of nitrogen contained in non-protein biochemicals.

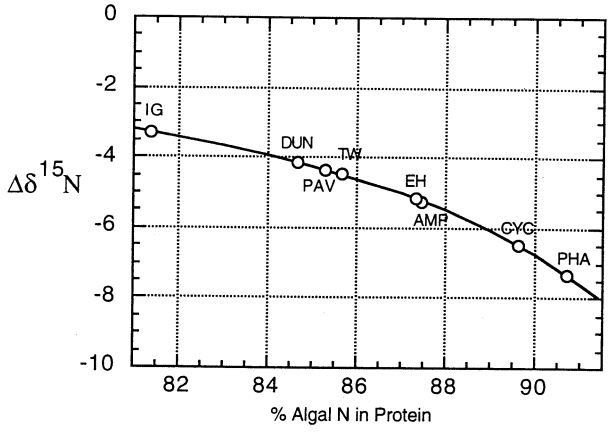


Fig. 5. Results of a simple model of ^{15}N distributions in phytoplankton. Differences in the isotopic depletion of chlorophyll *a* between algal species can be explained by interspecies variations in the partitioning of nitrogen into non-protein biochemicals.

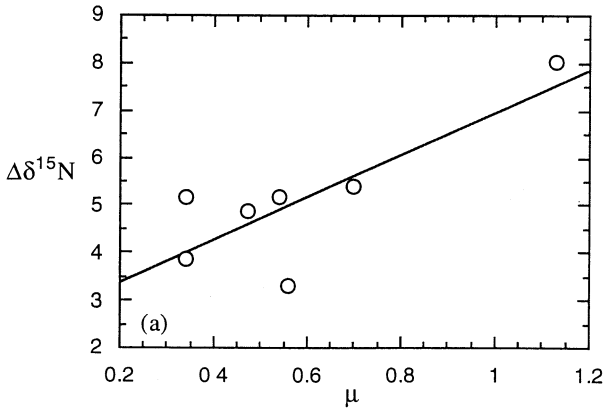
4.4. Growth Rate and Nitrogen Isotopic Fractionation

Another potential source of variation in the cell-chlorophyll isotopic difference is growth rate. Growth rate has been implicated as an important factor in the study of nitrogen (Wada and Hattori, 1978) and carbon (Goericke et al., 1994; Laws et al., 1995) isotopic fractionation in marine phytoplankton. It was shown to markedly affect whole-cell nitrogen isotopic fractionation during nitrate assimilation in certain marine diatoms (Montoya and McCarthy, 1995; Wada and Hattori, 1978). In those studies, larger isotopic fractionations were associated with slower growth rates.

We determined growth rates (μ) from the exponential part of the growth curve for 7 of the phytoplankton cultures grown in this study (Table 2). Values ranged from 0.34 day^{-1} for both a *Dunaliella tertiolecta* and an *Amphidinuim carterae* culture to 1.1 day^{-1} for a *Phaeodactylum tricornutum* culture. A linear regression of $\Delta\delta^{15}N_{cell\text{-}Chla}$ on growth rate indicates a moderate positive correlation between the two parameters ($r^2 = 0.7$; Fig. 6a). With the limited data, though, it is not possible to isolate the growth rate effect from the interspecies variation in $\Delta\delta^{15}N_{cell\text{-}Chla}$. Additional cultures of individual species grown at multiple rates are necessary to distinguish the 2 effects.

4.5. Nutrient Source and Chlorophyll-Cell N Isotopic Differences

The nitrogen species taken up by an algal cell may contribute to chlorophyll-cell isotopic differences, especially if exposure to different nutrient regimes and biosynthesis of specific nitrogen compounds are offset in time. Nitrogenous biochemicals synthesized in a nutrient-rich regime (i.e., the lower euphotic zone) may be depleted in ^{15}N as a result of kinetic isotope effects associated with dissolved inorganic nitrogen (DIN) uptake (Cifuentes et al., 1989; Goering et al., 1990; Horrigan et al., 1990; Montoya et al., 1991; Montoya and McCarthy, 1995; Wada, 1980; Wada and Hattori, 1978). Net isotopic fractionation should be small when nutrients are depleted (Altabet et al., 1991; Altabet and Francois, 1994; Francois and Altabet, 1992), as is the case in the upper euphotic zone. Furthermore,



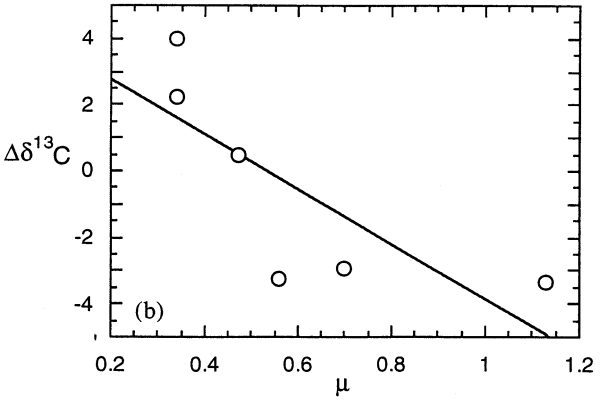


Fig. 6. The relationship between growth rate (μ) and the (a) nitrogen and (b) carbon isotopic difference between chlorophyll *a* and phytoplankton. Linear regressions of y on x yield the relationships: $\Delta\delta^{15}N_{cell\text{-}Chla} = 2.5 + 4.5\,\mu$, $r^2 = 0.7$ for nitrogen and $\Delta\delta^{13}C_{cell\text{-}Chla} = 4.4 - 8.2\mu$, $r^2 = 0.6$ for carbon.

the rate of chlorophyll biosynthesis is low (Goericke and Welschmeyer, 1992) in the upper euphotic zone, where light intensity and C:Chla (Cullen, 1982) ratios are high, and high at depth where the inverse is true. Therefore, a cell migrating from the lower to the upper euphotic zone would synthesize chlorophyll primarily when net cellular isotopic fractionation of nitrogen is high, leading to ^{15}N-depleted Chla, and other cellular components when net isotopic fractionation is low. This process would enhance the cell-chlorophyll isotopic difference.

However, 2 phenomena would counteract this effect. One is the observation that the phytoplankton biomass does not migrate vertically in the water column to a large extent (Forward, 1976). The second is our finding that $\Delta\delta^{15}N_{cell\text{-}Chla}$ is proportional to growth rate. Phytoplankton growth rates vary inversely with light intensity, and hence depth in the water column (Furnas, 1990; Goericke and Welschmeyer, 1998). Thus $\Delta\delta^{15}N_{cell\text{-}Chla}$ values should be lower in the low light regime at the base of the euphotic zone than at the surface. Since the range of $\Delta\delta^{15}N_{cell\text{-}Chla}$ values for low and high

Table 3. The nitrogen isotopic composition of chlorophyll and bulk particulate and sedimentary material in a wide diversity of settings. The average $\Delta\delta^{15}_{PON\text{-}Chla}$ for both particles and sediments is very close to that for cultured phytoplankton, −5.06‰, and reinforces the robustness of that relationship.

Location	Sample type (n)	$\delta^{15}N_{Chla}$	$\delta^{15}N_{PON}$	$\Delta\delta^{15}N_{PON\text{-}Chla}$
Eastern Mediterranean	Suspended particles (3)	−6.38	−0.63	5.75
Western Mediterranean	Suspended particles (3)	−2.52	2.22	4.74
Arabian Sea	Suspended particles (1)	5.57	9.1	3.53
Southern Ocean	Suspended particles (1)	2.52	6.7	4.18
Northwest Atlantic	Suspended particles (1)	−5.38	3.7	9.08*
Eastern Equat. Pacific	Suspended particles (1)	1.77	6.1	4.33
	Average for Particles			**5.27**
Eastern Mediterranean	Sapropel sediments (7)	−5.01	−0.08	4.93
Black Sea Unit I	0–10 cm sediment (1)	−4.51	1.3	5.81
Black Sea Unit II	Sapropel sediment (1)	−5.8	0.45	6.25
Peru Margin	2–10 cm Sediment (3)	2.27	6.83	4.56
	Average for Sediments			**5.39**

* The Georges Bank sampling site was very shallow (~20 m water depth) and characterized by intense vertical mixing. It is likely that the suspended PON sample contained a significant component of resuspended detritus, leading to an elevated $\delta^{15}N$ value.

growth rates (fig. 6a) is comparable to the observed fractionation factors associated with DIN uptake by phytoplankton (Cifuentes et al., 1989; Goering et al., 1990; Horrigan et al., 1990; Montoya et al., 1991; Montoya and McCarthy, 1995; Wada, 1980; Wada and Hattori, 1978), the 2 effects may offset one another in the ocean.

4.6. Nitrogen Isotopic Results from the Field

Our field results support the finding from cultures of a ca. 5.1‰ depletion in chlorophyll relative to total algal nitrogen. A comparison of the $\delta^{15}N$ value of Chla and suspended particulate matter were made in 6 ocean basins spanning a wide range of nutrient regimes and rates of primary productivity: the Eastern and Western Mediterranean Sea, Arabian Sea, Southern Ocean, Northwest Atlantic, and Eastern Equatorial Pacific. In this diversity of locations chlorophyll *a* isotopic values ranged from −6.38 to 5.57‰, while PON $\delta^{15}N$ values ranged from −0.63 to 9.1‰ (Table 3). The highest $\delta^{15}N$ values for both Chla and PON were in the Arabian Sea where denitrification enriches the DIN pool in ^{15}N (Altabet et al., 1995). Irrespective of the absolute $\delta^{15}N$ values, though, the PON-chlorophyll isotopic difference was relatively constant, averaging 5.27 ± 1.48‰. This average from the field is very close to that from cultures (5.06 ± 1.13‰), especially in light of the fact that suspended particles contain heterotrophs and detritus known to have different N isotopic signatures than phytoplankton (Fry, 1988).

Results from sediments underlying suboxic to anoxic water, where the isotopic composition of sedimenting particles is preserved (Sachs, 1997), also support the findings from phytoplankton cultures. A comparison of the $\delta^{15}N$ value of Chla and bulk sediment was made in 6 Late Pleistocene Eastern Mediterranean sapropels (Sachs and Repeta, subm.), Peru Margin surface sediments, surficial Black Sea sediments, and the Holocene sapropel from the Black Sea. Nitrogen isotopic values ranged from −5.8 to 2.27‰ in Chla, and from −0.08 to 6.83‰ in bulk sediments (Table 3). The sediment-Chla isotopic difference averaged 5.39 ± 0.67‰ in these locations, a value close to those from suspended particles (5.27‰) and cultures (5.06‰), and provides another indication that the 5.1‰ isotopic depletion of chlorophyll relative to total algal nitrogen is a robust result.

4.7. Carbon Isotopic Differences between Chlorophyll and Algae

Twelve culture experiments with 7 species of marine phytoplankton indicate that chlorophyll is isotopically similar to total cellular carbon, the average $\Delta\delta^{13}C_{cell\text{-}Chla}$ being −0.32 ± 1.61‰ (fig. 3). This compares with published $\Delta\delta^{13}C_{plant\text{-}Chla}$ values (Table 2) of −1.6 ± 1.4‰ ($n = 7$: 6 terrestrial plants, 1 phytoplankton species; Kennicutt II et al., 1992) and 2.1 ± 1.2‰ ($n = 3$: 1 vascular plant, 1 macroalga and 1 phytoplankton species; Galimov and Shirinsky, 1975). The large variation around the mean $\Delta\delta^{13}C_{plant\text{-}Chla}$ values in all 3 studies suggests that substantial inter-species differences in $\Delta\delta^{13}C_{cell\text{-}Chla}$ may exist. However, we cannot rule out the possibility that the cell-chlorophyll carbon isotopic difference in phytoplankton from this study results primarily from environmental conditions since results of ANOVA on the seven species groupings indicate no significant ($P > 0.05$) inter-species variance that cannot be explained by within-species variance.

A likely cause for observed variance in $\Delta\delta^{13}C_{cell\text{-}Chla}$ between algal cultures is growth rate and its potential influence on isotopic heterogeneity in the chlorophyll *a* molecule. Estimates of growth rate were made for 6 of the phytoplankton cultures for which $\Delta\delta^{13}C_{cell\text{-}Chla}$ data are available (Table 2). A linear regression of $\Delta\delta^{13}C_{cell\text{-}Chla}$ on μ indicates a moderate negative correlation between the 2 parameters ($r^2 = 0.6$; Fig. 6b). Slow-growing phytoplankton (0.3 to 0.5 day^{-1}) contained Chla depleted in ^{13}C relative to total cellular carbon by up to 4.0‰, while rapidly growing phytoplankton (0.6 to 1.1 day^{-1}) contained Chla enriched in ^{13}C by up to 3.4‰ relative to total cellular carbon.

These differences may result partly from the source of carbon for the 2 components of the chlorophyll *a* molecule (Fig. 4). The C_{20} isoprenoid side chain (phytol) (Rudiger and Schoch, 1991), and the C_{35} cyclic tetrapyrrole (macrocycle) of chlorophyll *a* (Beale and Weinstein, 1991) derive carbon from

different biosynthetic pathways. The Chla macrocycle derives isotopically enriched carbon (Abelson and Hoering, 1961) from the tricarboxylic acid (TCA) cycle. Phytol, a lipid, is depleted in ^{13}C relative to total cellular carbon (DeNiro and Epstein, 1977; Galimov and Shirinsky, 1975). However, the source of carbon to the TCA cycle alternates between more isotopically enriched oxaloacetic acid (O'Leary et al., 1981) when cell growth is rapid, and less isotopically enriched acetyl-Coenzyme A (acetyl CoA; DeNiro and Epstein, 1977) when cell growth is slow. Growth rate, a function of the culturing conditions (i.e., light intensity, pH, nutrient concentration), is thus expected to alter the isotopic difference between chlorophyll and total cellular carbon as ratios of carbon-flow at key biosynthetic branch points change.

A carbon isotopic depletion of 1.6 to 5.1‰ in phytol relative to the macrocycle has been reported from nettle leaves (Bogacheva et al., 1979). Also reported was a −0.01 to 0.73‰ carbon isotopic enrichment of the chlorin macrocycle relative to whole algal cells and a 0.66‰ enrichment compared to beech tree leaves (Hayes et al., 1987; and references therein). Incorporating these ranges of values into a mass balance indicates a range of $\Delta\delta^{13}C_{cell\text{-}Chla}$ from the literature of −1.86 to +0.15‰. Although the average $\Delta\delta^{13}C_{cell\text{-}Chla}$ value measured in our study (−0.32‰) falls within this range, many of the individual analyses lie outside (fig. 3), as do all 3 measurements made by Galimov and Shirinsky (1975) and one third (e.g., 2 of 6) of the measurements made by Kennicutt el al. (1992). We hypothesize that non-steady state environmental conditions resulted in growth rate differences within and between these experiments, and that such differences are the likely cause of the wide range of $\Delta\delta^{13}C_{cell\text{-}Chla}$ values observed.

5. CONCLUSION

Culture experiments with 8 species of marine phytoplankton indicate that chlorophyll *a* is depleted in ^{15}N by a nearly constant amount relative to total cellular nitrogen. This quantity, 5.06 ± 1.13‰ (95% confidence interval), is similar to results from field comparisons between chlorophyll *a* and bulk PON $\delta^{15}N$ values in suspended particles (5.27 ± 1.48‰ (1σ); $n = 6$) and sub-oxic to anoxic sediments (5.39 ± 0.67‰ (1σ); $n = 4$) from a wide diversity of locations. Both field and laboratory studies thus indicate that the 5.1‰ isotopic depletion of chlorophyll relative to total algal nitrogen is a robust result. Phytoplankton $\delta^{15}N$ values can therefore be determined from particulate and sedimentary chlorophyll *a* by the addition of 5.1‰.

Much of the nitrogen isotopic depletion of chlorophyll *a* relative to total cellular nitrogen probably occurs during the transamination of glutamic acid in δ-aminolevulinic acid biosynthesis, the first committed precursor to chlorophyll. Disparity in the partitioning of nitrogen between protein and other nitrogenous biochemicals is a likely cause for the observed small interspecies variations in $\Delta\delta^{15}N_{cell\text{-}Chla}$. Growth rate also has a small effect on $\Delta\delta^{15}N_{cell\text{-}Chla}$ and likely accounts for some of the intra-species variation in the cell-chlorophyll isotopic difference. However, as the field experiments indicate, the factors causing intra- and inter-species variation in $\Delta\delta^{15}N_{cell\text{-}Chla}$ of monospecific batch cultures are probably minmized in the ocean where multiple species and growth rates always occur in the same water column.

The carbon isotopic composition of chlorophyll from 12 cultures and 7 species of marine phytoplankton was elevated by 0.32 ± 1.61‰ (95% confidence interval) relative to total cellular carbon. No significant interspecies variance was observed. There was a moderate inverse correlation between growth rate and $\Delta\delta^{13}C_{cell\text{-}Chla}$, and it is hypothesized that this parameter is largely responsible for the large range of intra-species $\Delta\delta^{13}C_{cell\text{-}Chla}$ values observed. Growth rate dictates the source of carbon to the tricarboxylic acid cycle that supplies carbon to the chlorophyll *a* macrocycle. Isotopically enriched oxaloacetic acid is supplied to the TCA cycle when cell growth is rapid, while ^{13}C-depleted acetyl-Coenzyme A is used when growth is slow.

Stable isotopic measurements on individual compounds are increasingly common in geochemical and paleoenvironmental studies. Whereas isotopic values of bulk phases often integrate a complex sequence of accretive and consumptive processes, each with the potential to modify these values, biomarkers allow isotopic determinations of a definitive biological source. Nitrogen isotopic alteration during decomposition processes is notably severe and can be at least as large as the isotopic diversity of nitrogen inputs to a system. Chlorophyll is ubiquitous in photoautotrophs and can serve as an isotopic benchmark for primary producers.

Acknowledgments—We would like to thank Brian Fry, Kris Tholke and Bob Michener for performing and assisting with isotopic analyses. We are also grateful for the assistance and advice on the culturing of marine phytoplankton provided by Joe Montoya, Don Anderson and Dave Kulis. John Waterbury graciously provided the *Synechococcus* culture.

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