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Paleoenvironmental implications of taxonomic variation among $\delta^{15}N$ values of chloropigments

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Abstract

Natural variations in the ratios of nitrogen isotopes in biomass reflect variations in nutrient sources utilized for growth. In order to use $\delta^{15}N$ values of chloropigments of photosynthetic organisms to determine the corresponding $\delta^{15}N$ values of biomass – and by extension, surface waters – the isotopic offset between chlorophyll and biomass must be constrained. Here we examine this offset in various geologically-relevant taxa, grown using nutrient sources that may approximate ocean conditions at different times in Earth's history. Phytoplankton in this study include cyanobacteria (diazotrophic and non-diazotrophic), eukaryotic algae (red and green), and anoxygenic photosynthetic bacteria (Proteobacteria), as well as environmental samples from sulfidic lake water. Cultures were grown using N_2, NO_3^-, and NH_4^+ as nitrogen sources, and were examined under different light regimes and growth conditions. We find surprisingly high variability in the isotopic difference ($\delta^{15}N_{biomass} - \delta^{15}N_{chloropigment}$) for prokaryotes, with average values for species ranging from −12.2‰ to +11.7‰. We define this difference as ε_{por}, a term that encompasses diagenetic porphyrins and chlorins, as well as chlorophyll. Negative values of ε_{por} reflect chloropigments that are ^{15}N-enriched relative to biomass. Notably, this enrichment appears to occur only in cyanobacteria. The average value of ε_{por} for freshwater cyanobacterial species is -9.8 ± 1.8‰, while for marine cyanobacteria it is -0.9 ± 1.3‰. These isotopic effects group environmentally but not phylogenetically, e.g., ε_{por} values for freshwater Chroococcales resemble those of freshwater Nostocales but differ from those of marine Chroococcales. Our measured values of ε_{por} for eukaryotic algae (range = 4.7–8.7‰) are similar to previous reports for pure cultures. For all taxa studied, values of ε_{por} do not depend on the type of nitrogen substrate used for growth. The observed environmental control of ε_{por} suggests that values of ε_{por} could be useful for determining the fractional burial of eukaryotic vs. cyanobacterial organic matter in the sedimentary record.

1. INTRODUCTION

Nitrogen isotopes in the sedimentary record can serve as a useful proxy for marine nutrient cycling. The biomass of primary producers acts as an integrated signal of phytoplankton nutrient sources in marine surface waters. Because diagenetic reactions, both before and after burial, may unpredictably alter the isotopic signal of this biomass

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(Sachs and Repeta, 1999; Sigman et al., 1999; Robinson et al., 2005), one way to avoid potential problems in interpretation of sedimentary isotope data is to measure isolated organic nitrogen pools that are not affected by diagenesis (Chicarelli et al., 1993; Sachs and Repeta, 1999; Sachs et al., 1999; Sigman et al., 1999; Robinson et al., 2004, 2005; Chikaraishi et al., 2008; Kashiyama et al., 2008a,b, 2010; Higgins et al., 2009, 2010; Ren et al., 2009).

Compound-specific $\delta^{15}N$ measurement of chlorophyll biomarkers is an especially attractive option, because the light-harvesting pigments chlorophyll or bacteriochlorophyll are produced by all photosynthetic organisms and therefore provide a record of surface water processes. Since degradation of the tetrapyrrole macrocycle occurs initially at C–C methine bonds, measuring intact chlorins and porphyrins ensures a nitrogen isotopic value unaltered by diagenesis (Louda and Baker, 1986). Recent advances in analytical techniques have enabled the measurement of minute quantities of chlorins and porphyrins, rendering these methods applicable for paleoceanographic studies on even low total organic carbon (TOC) sediments (Chikaraishi et al., 2008; Kashiyama et al., 2008b, 2010; Higgins et al., 2009, 2010). However, these proxies can be used to constrain paleoceanographic interpretations only if the relationship between chloropigment and total biomass $\delta^{15}N$ values is well understood across phylogenetically diverse phytoplankton, including eukaryotic phytoplankton, as well as aerobic and anaerobic photosynthetic bacteria.

Several studies have examined the nitrogen isotope fractionation between cultured organisms and chlorophyll, ε_{por} ($\varepsilon_{por} = \delta^{15}N_{biomass} - \delta^{15}N_{chloropigment}$; Fig. 1). These studies have focused mostly on eukaryotic taxa that are dominant in modern marine systems, as well as on terrestrial plants (Sachs, 1997; Sachs et al., 1999; Kennicutt et al., 1992). Sachs et al. (1999) measured an average value of $\varepsilon_{por} = 5.1 \pm 1.1‰$ (*i.e.*, chlorophyll was ~5‰ depleted in ^{15}N relative to biomass) in seven species of eukaryotic phytoplankton and one cyanobacterium (*Synechococcus* sp., order Chroococcales). To date, two studies have focused on ε_{por} values specifically in bacteria. Beaumont et al. (2000) measured values of ε_{por} for *Rhodobacter capsulatus* (Alphaproteobacteria, purple non-sulfur) and *Anabaena cylindrica* (Cyanobacteria, order Nostocales) grown on different nitrogen and carbon sources (*Anabaena*: N_2 and CO_2, *Rhodobacter*: N_2 vs. NH_4^+, CO_2 vs. malate). Values of ε_{por} for *Rhodobacter* averaged $8.6 \pm 1.6‰$. Strikingly, values of ε_{por} for *Anabaena* averaged $-8.5 \pm 0.7‰$; *i.e.*, chlorophyll was enriched in ^{15}N relative to total biomass. This result is similar to an earlier report in which Katase and Wada (1990) measured ε_{por} values between −12.9 and −16.0‰ in environmental samples of *Mycrocystis* spp. (Cyanobacteria, order Chroococcales) isolated from a Japanese lake. Together these data suggest there may be fundamentally different controls on values of $\delta^{15}N_{chlorophyll}$ in cyanobacteria.

In this study we investigate the taxonomic distribution of ε_{por} values in order to aid in the interpretation of compound-specific nitrogen isotope data from the sedimentary record. We specifically examine eukaryotic and prokaryotic phytoplankton that are relevant for paleoceanographic studies of different times in Earth history. Included are seven species of cyanobacteria, two species of anoxygenic photosynthetic bacteria, and two eukaryotic algae (chlorophyte and rhodophyte). Batch cultures were grown on different N sources (NH_4^+, NO_3^- and N_2), and light conditions (continuous light vs. a diel light:dark cycle). In many of the cultures, values of ε_{por} were determined at multiple time points during growth (lag, log and stationary phase). Our results are consistent with prior observations and confirm that values of ε_{por} in cyanobacteria are fundamentally different in comparison to other photosynthetic taxa.

2. METHODS

2.1. Culture and growth conditions

Cultures included *Synechocystis* sp. PCC 6803, *Synechococcus* sp. WH8102, *Nostoc* sp. PCC 7120, *Anabaena*

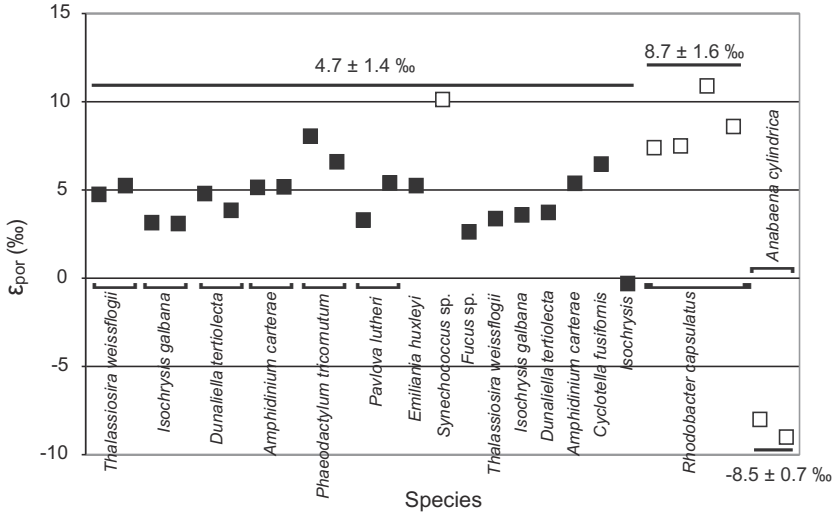

Fig. 1. Previously published $\varepsilon_{por} = \delta^{15}N_{biomass} - \delta^{15}N_{chlorophyll}$ data, taken from Sachs et al., 1999; Kennicutt et al., 1992; Beaumont et al., 2000. Hollow squares represent photosynthetic bacteria, and filled squares represent eukaryotic phytoplankton.

variabilis ATCC 29413, *Crocosphaera watsonii* WH8501, *Prochlorococcus marinus* MED4 (from S. Chisholm), *Rhodopseudomonas palustris* CGA009 (from T. Bosak), and *Chlamydomonas reinhardtii* CC125. *Porphyridium cruentum* (CCMP 1328) was obtained from the Bigelow Laboratory Center for Culture of Marine Phytoplankton as a 50 mL live culture and sampled directly. The *Phormidium* mat-forming cyanobacterial culture (obtained from T. Bosak) was previously isolated from Yellowstone microbial mats and cultured according to Bosak et al. (2009).

Aerobic cultures were maintained axenically in 1 L or 500 mL acid-cleaned polycarbonate bottles, continuously aerated (except *Crocosphaera*) with sterile-filtered air, and illuminated by fluorescent cool-white lamps under the irradiance and temperature conditions as indicated in Table EA-1-1. *Synechocystis* sp., *Nostoc* sp., *A. variabilis*, and *R. palustris* were grown on modified BG-11 medium containing 1.8 mM NH_4^+ (Allen and Stanier, 1968). If necessary, pH was adjusted to 8.3 with MES (2-(*N*-Morpholino)ethanesulfonic acid) as an organic buffer. *C. reinhardtii* was grown on modified TP minimal medium with 7.5 mM NH_4^+ (Harris, 1989). *Synechococcus* sp. was grown on SN media containing either 9 mM NO_3^- or 1 mM NH_4^+ (Waterbury et al., 1986). *Prochlorococcus* was grown on Pro99ESL media containing 0.8 mM NH_4^+, under a 14:10 light–dark cycle, as described by Zinser et al. (2009). *Porphyridium* was grown on a 13:11 L:D cycle, using f/2-Si media containing 0.88 mM NO_3^- (Guillard, 1960). Axenic cultures of *C. watsonii* were grown in nitrogen-free SO medium at 28 °C under a 14:10 light–dark cycle (Waterbury et al., 1986; Webb et al., 2001).

2.2. Environmental sample

An environmental sample of *Lamprocystis purpurea* (formerly called *Amoebobacter purpureus*) was obtained *in situ* from Mahoney Lake, British Columbia, Canada. Mahoney Lake is a meromictic lake characterized by a high density of anoxygenic purple sulfur bacteria in the chemocline (Overmann et al., 1991). This layer contains bacteriochlorophyll *a* concentrations of up to 20,900 μg/L, and is dominated (>98% of photosynthetic cells) by *L. purpurea*, a member of the Gammaproteobacteria. Suspended cells were collected from the chemocline at a depth of 7 m and frozen until analysis.

2.3. Growth and pigment analyses

Whole-cell spectral measurements from 400 to 800 nm were made on a Beckman Coulter DU-640 spectrophotometer. For the cyanobacteria, chlorophyll *a* (Chl *a*) concentrations were determined using 100% methanol (MeOH) extracts of biomass pellets collected in duplicate from 2 mL of culture, centrifuged at 21,000*g* for 10 min at 4 °C. The absorbance of these extracts was analyzed at 665 nm and Chl *a* concentration was calculated according to Porra (2002). The concentrations of Chl *a* and Chl *b* were measured with 90% acetone extracts of *Chlamydomonas* biomass (centrifuged from 2 mL culture aliquots) at 646 and 653 nm (Jeffrey and Humphrey, 1975; Porra, 2002). Chlorophyll concentrations were monitored daily throughout growth experiments.

2.4. Sample processing and chlorophyll extraction

Cultures grown under continuous light were sampled at specific times during the growth cycle. Continuous-light cultures of *Synechocystis* grown on NO_3^-, *Nostoc* grown on NO_3^- and N_2, *Anabaena* grown on NO_3^-, NH_4^+, and N_2, and *Chlamydomonas* grown on NH_4^+, were sampled daily in duplicate 1 mL aliquots. For continuous-light grown cultures of *Synechococcus*, *Nostoc*, and *Rhodopseudomonas* with NH_4^+, duplicate 50 mL aliquots of culture were taken every other day. Cultures grown under diel-light conditions were sampled in duplicate 50 mL aliquots during log phase, during several serial dilutions. For each diel sample, duplicate aliquots were taken during consecutive light and dark periods. The specific days along the growth curve at which cultures were sampled are indicated in Supplemental Figures EA-1-1 and EA-1-2.

Cell pellets from cultures of *Synechocystis* grown on NO_3^-, *Nostoc* grown on NO_3^- and N_2, *Anabaena* grown on NO_3^-, NH_4^+, and N_2, and *Chlamydomonas* grown on NH_4^+ were centrifuged in 1.5 mL centrifuge tubes, decanted, washed and pellets were frozen at −20 °C. Samples grown on NH_4^+ and NO_3^- were gently washed after thawing. All other cultures were washed twice by resuspension in fresh, sterile N-free medium, followed by centrifugation in 50 mL or 250 mL clean conical centrifuge tubes. The supernatant was removed, and the cell pellets were flash frozen with liquid N_2 and stored at −80 °C.

Cell pellets were extracted using a 2:1 (v/v) mixture of dichloromethane (DCM)/MeOH with vortexing (1 min), sonication (10 min), and incubation in the dark for >2 h at 4 °C. Silica gel columns were prepared by adding glass wool, Na_2SO_4, and SiO_2 gel to a 5" glass pipette, and then combusted. Sample extracts were transferred to the columns, eluted with 1 mL 2:1 DCM/MeOH, and stored at −20 °C until analysis. All glassware was precombusted, and organic solvents were all GC^2 grade.

Each extract was analyzed for total Chl *a* or bacteriochlorphyll *a* (Bchl *a*) by HPLC (Agilent 1100 series with multi-wavelength UV/Vis detector). Samples were injected to a ZORBAX SIL column (4.6 × 25 mm, 5 μm), and eluted using a gradient from hexane to 50:50 MeOH/EtOAC (v/v; Table EA-1-2), at 1 mL/min. Combined extracts containing an estimated ~50 nmol N were evaporated and brought up to 50 μL in DCM. In cases where N contents were lower than 30 nmol N/sample, consecutively-sampled cell pellets (adjacent time points) were combined to yield enough N for isotopic analysis. Pigments were collected using the same HPLC program, with time-based fraction collection for Chl *a* or Bchl *a* peak elution (usually 6–16 and 5–15 min, respectively).

Chl *a* concentrations were determined from integrated absorbance at 410 nm, using a conversion factor (4.25e-3 nmol Chl* $mAU^{-1}min^{-1}$) that was calculated using the same HPLC program for Chl *a* standard (Sigma). The conversion factor for Bchl *a* was empirically determined by comparing HPLC absorbances at 364 and 770 nm of

Rhodopseudomonas Bchl samples to their N concentrations after oxidation.

2.5. Chlorophyll isotopic analysis

Chl and Bchl were quantitatively converted to NO_3^- according to the methods outlined in Higgins et al. (2009). Briefly, samples were oxidized in quartz tubes, followed by chemical oxidation using 0.05 M $K_2S_2O_8$ that was recrystallized three times and dissolved in fresh 0.15 M NaOH. Nitrate concentration was measured using a chemiluminescent analyzer (Monitor Labs) and $\delta^{15}N$ values were measured on samples containing 10 nmol N using the denitrifier method with *Pseudomonas chlororaphis* (Sigman et al., 2001; Casciotti et al., 2002) on a Thermo Scientific Delta V isotope ratio mass spectrometer. Isotopic measurements were standardized to the N_2 reference scale using the KNO_3 standard IAEA N3. Values of $\delta^{15}N$ were corrected for N blank according to Higgins et al. (2009). Oxidizing reagent N concentrations were measured, and assigned a $\delta^{15}N$ value of $5 \pm 10‰$ (based on measured values; data not shown). HPLC solvent N concentrations were estimated according to Higgins et al. (2009) and assigned a $\delta^{15}N$ value of $0 \pm 10‰$.

2.6. Bulk isotopic analysis

Thawed cell pellets were resuspended in nanopure water, vortexed, and aliquoted into clean centrifuge tubes at a volume corresponding to a final total mass of ~2 μmol cell N. In cases where Chl N concentrations were low enough that samples had to be combined for $\delta^{15}N_{chl}$ analysis, cell samples were combined in the same ratio for bulk $\delta^{15}N$ analysis before aliquoting. Aliquots for bulk isotopic analysis were transferred 100 μL at a time to tin capsules that each contained a small piece of combusted glass fiber filter, and then desiccated. Dry capsules were folded and crushed, and analyzed on a Carlo Erba elemental analyzer connected to a Delta Plus isotope ratio mass spectrometer (Thermo Finnigan) or a Costech 4010 Elemental Analyzer connected to a Delta V (Thermo). Sample $\delta^{15}N$ values were calculated using standards IAEA1 and IAEA2.

3. RESULTS AND DISCUSSION

3.1. Fractionations associated with chlorophyll synthesis in eukaryotes

Cultures of the chlorophyte (primary green) alga *C. reinhardtii* were analyzed for the effects of growth rate and light conditions on the N isotopic difference between chlorophyll and biomass (Figure EA-1-1). In *Chlamydomonas* cultures grown on NH_4^+, ε_{por} values averaged $6.6 \pm 1.3‰$ when grown under continuous light, and $6.6 \pm 1.4‰$ when grown under a diel light regime. The magnitude of the *Chlamydomonas* ε_{por} values increased along the growth curve, starting at $5.6 \pm 0.9‰$ on days 4–6, falling briefly to $4.9 \pm 0.6‰$ on day 8, and increasing to $8.7 \pm 0.6‰$ on day 18 (Fig. 2, Table 1). The overall increase in ε_{por} values across the experiments may reflect the effects of nitrogen limitation on the partitioning of amino acids used as precursors for chlorophyll biosynthesis, and/or the effects of nitrogen limitation on the culture (see discussion below in Section 3.3.1).

Similar fractionation values were seen for a eukaryotic primary red (rhodophyte) alga (Fig. 2). The ε_{por} value measured for *P. cruentum* grown with NO_3^- under diel conditions was $6.8 \pm 1.1‰$. In comparison to other data from the literature (Fig. 1), most of which are from more recently-evolving heterokont algae (Falkowski and Knoll, 2007), our results support the idea that nitrogen isotopic fractionation in chlorophyll biosynthesis is similar across the breadth of eukaryotic phytoplankton, with average values of ε_{por} predictably between 5 and 7‰.

3.2. Fractionations associated with bacteriochlorophyll synthesis in anoxygenic photosynthetic bacteria

To examine fractionation factors for taxa that are important in anaerobic systems, we measured ε_{por} values for two anoxygenic photosynthetic bacteria. An environmental sample of the purple sulfur Gammaproteobacterium *L. purpurea* was collected from sulfidic lake water. *L. purpurea* has an ε_{por} value of $5.8 \pm 0.6‰$, similar to values measured for eukaryotic algae (Fig. 2). This is an expected result, because bacteriochlorophyll synthesis in Gammaproteobacteria

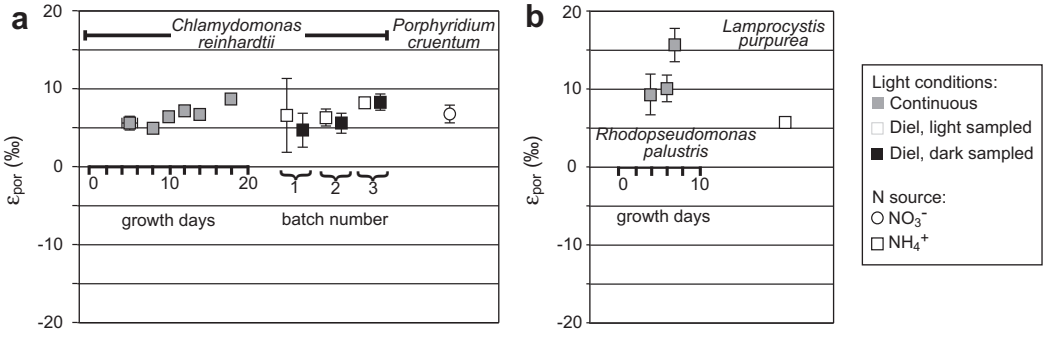

Fig. 2. Measured values of ε_{por} for eukaryotic algae (a) and anoxygenic bacteria (b). Shapes indicate N species supplied for growth, and shading distinguishes light regime. The growth days of the cultures at the time of sampling are shown on the *x*-axis for continuous light samples; else the designation (light or dark) and batch numbers are shown for diel samples. Growth data are shown in Figure EA-1-1, and ε_{por} as a function of growth rate is shown in Figure EA-1-1–3.

Table 1
Average $\delta^{15}N$ data. All isotope values are shown as average ±1 S.D. when multiple measurements were made, except in the case of $\varepsilon_{por} = \delta^{15}N_{biomass} - \delta^{15}N_{chlorophyll}$, where the error is calculated by compounding the errors of $\delta^{15}N_{chlorophyll}$ and $\delta^{15}N_{biomass}$.

Species	Light regime	N source $\delta^{15}N$	Growth days	[Chl *a*] (μg/ml)	μ (d^{-1})	$\delta^{15}N_{chl}$		$\delta^{15}N_{cell}$		ε_{por}	
						Day	Night	Day	Night	Day	Night
Anabaena variabilis ATCC 29413	Continuous	NO_3^-	4–7	2.2 ± 0.3	0.59	10.0 ± 0.5		2.7 ± 0.1		−7.4 ± 0.5	
		6.1 ± 0.3	8–9	5.1 ± 0.1	0.23	10.1 ± 1.1		2.2 ± 0.2		−8.0 ± 1.2	
			10	5.7 ± 0.5	−0.05			2.5 ± 0.2			
		NH_4^+	4–7	1.4 ± 0.1	0.52	7.7 ± 0.7		−1.1 ± 0.2		−8.8 ± 0.8	
		1.9 ± 0.1	9–10	4.7 ± 1.4	0.23	6.6 ± 0.5		−1.2 ± 0.2		−7.8 ± 0.5	
		N_2	4–8	1.6 ± 1.0	0.48	6.8 ± 1.0		−1.1 ± 0.1		−7.9 ± 1.0	
		0.7 ± 0.7	9–10	4.2 ± 0.3	0.08	6.5 ± 0.9		−0.9 ± 0.3		−7.3 ± 1.0	
Nostoc sp. PCC 7120	Continuous	NO_3^-	5–8	2.6 ± 0.8	0.24	6.0 ± 0.1		−1.6 ± 0.1		−7.6 ± 0.1	
		6.1 ± 0.3	9–10	3.1 ± 0.1	0.07	3.3 ± 0.6		−2.4 ± 0.1		−5.7 ± 0.6	
		NH_4^+	3	0.8 ± 0.3	0.24	−2.0 ± 0.2		−10.1 ± 0.1		−8.1 ± 0.2	
		−0.1 ± 0.1	5	1.8 ± 0.1	1.05	14.7 ± 0.3		2.9 ± 0.4		−11.8 ± 0.5	
			7	3.8 ± 0.2	0.18	18.3 ± 0.3		4.5 ± 0.1		−13.8 ± 0.4	
			8	4.1 ± 0.1	0.09	16.6 ± 0.1		4.5 ± 0.0		−12.2 ± 0.2	
			11	4.6 ± 0.1	0.10	14.5 ± 2.1		1.8 ± 0.2		−12.7 ± 2.1	
		N_2	5–8	3.7 ± 3.0	0.63	7.6 ± 0.1		−1.1 ± 0.8		−8.7 ± 0.8	
		0.7 ± 0.7	9	6.5 ± 0.8	−0.25	–		−0.2 ± 0.7		–	
			10	5.8 ± 0.2	−0.11	6.7 ± 0.3		−0.2 ± 0.3		−6.9 ± 0.4	
	Diel (12/12)	NO_3^-	n/a	3.1 ± 0.3	0.15	−8.1 ± 0.8	−5.2 ± 0.9	−17.1 ± 1.3	−16.0 ± 2.6	−8.9 ± 1.5	−10.8 ± 2.7
		4.3 ± 0.9	n/a	3.6 ± 0.1	0.25	−6.0 ± 0.9	−3.9 ± 0.5	−15.1 ± 1.5	−14.9 ± 1.4	−9.0 ± 1.7	−11.0 ± 1.5
			n/a	7.5 ± 0.5	0.13	–	3.5 ± 0.4	−6.1 ± 0.4	−5.9 ± 0.4	–	−9.4 ± 0.5
Synechocystis sp. PCC 6803	Continuous	NO_3^-	7–8	12.9 ± 0.6	0.42	12.4 ± 1.3		2.6 ± 0.2		−9.8 ± 1.3	
		6.1 ± 0.3	10–11	25.5 ± 3.8	0.18	13.5 ± 1.6		3.2 ± 0.2		−10.3 ± 1.7	
			14–15	–	–	13.7 ± 0.6		3.9 ± 0.0		−9.8 ± 0.6	
			18	–	–	12.5 ± 0.8		4.9 ± 0.0		−7.6 ± 0.8	
		NH_4^+	3	1.4 ± 0.4	0.52	10.7 ± 0.7		−4.4 ± 0.1		−15.1 ± 0.7	
		−0.1 ± 0.1	5	2.6 ± 0.2	0.31	19.8 ± 0.8		4.9 ± 0.1		−14.9 ± 0.8	
			7	3.0 ± 0.0	0.07	18.4 ± 1.4		4.9 ± 0.0		−13.5 ± 1.4	
			8	2.7 ± 0.1	−0.10	17.3 ± 3.6		4.9 ± 0.1		−12.5 ± 3.6	
	Diel (12/12)	NO_3^-	n/a	1.3 ± 0.2	0.21	–	–	−2.2 ± 0.6	−1.9 ± 0.8	–	–
		4.3 ± 0.9	n/a	3.1 ± 0.2	0.29	12.7 ± 1.0	10.7 ± 1.0	−1.0 ± 0.3	−2.0 ± 1.2	−13.7 ± 1.0	−12.6 ± 1.6
			n/a	2.8 ± 0.2	0.25	8.0 ± 0.8	8.7 ± 1.1	−3.1 ± 0.2	−3.1 ± 0.7	−11.1 ± 0.8	−11.9 ± 1.3
			n/a	5.3 ± 0.4	0.29	10.5 ± 0.7	12.5 ± 0.9	−2.8 ± 0.5	−2.6 ± 0.0	−13.3 ± 0.9	−15.1 ± 0.9

(*continued on next page*)

Table 1 (*continued*)

Species	Light regime	N source $\delta^{15}N$	Growth days	[Chl *a*] (μg/ml)	μ (d^{-1})	$\delta^{15}N_{chl}$		$\delta^{15}N_{cell}$		ε_{por}	
						Day	Night	Day	Night	Day	Night
Synechococcus sp. WH8102	Continuous	NO_3^-	3–5	0.7 ± 0.2	0.38	0.3 ± 0.2		0.8 ± 0.4		0.6 ± 0.4	
		5.7 ± 1.1	9	2.2 ± 0.1	0.31	1.1 ± 0.2		0.5 ± 0.3		−0.6 ± 0.3	
			11	2.9 ± 0.2	0.08	1.5 ± 1.0		0.6 ± 0.5		−0.9 ± 1.1	
			13	4.7 ± 0.1	0.26	1.9 ± 1.1		0.7 ± 0.6		−1.2 ± 1.3	
		NH_4^+	2–4	0.3 ± 0.1	0.84	−8.6 ± 2.8		−7.1 ± 1.1		1.5 ± 3.0	
		3.3 ± 0.6	5	0.9 ± 0.0	0.67	0.3 ± 1.3		−1.4 ± 0.8		−1.7 ± 1.5	
			7	1.3 ± 0.1	0.10	4.7 ± 1.2		4.1 ± 0.9		−0.6 ± 1.5	
			13	0.7 ± 0.0	−0.28	6.3 ± 1.4		3.4 ± 0.2		−2.9 ± 1.4	
	Diel (12/12)	NO_3^-	n/a	0.7 ± 0.1	0.14			−25.2 ± 6.0	−22.7 ± 7.9		
		5.7 ± 0.1	n/a	1.2 ± 0.1	0.19	−21.3 ± 1.4	−18.5 ± 2.4	−17.5 ± 2.7	−19.2 ± 0.2	3.8 ± 3.1	−0.6 ± 2.4
			n/a	2.9 ± 0.1	0.04	−14.9 ± 2.4	−12.7 ± 2.1	−9.1 ± 0.9	−8.4 ± 0.6	5.7 ± 2.6	4.3 ± 2.2
Crocosphaera watsonii	Diel (14/10)	N_2	n/a			0.1 ± 1.2	0.0 ± 0.6	−1.3 ± 0.4	−1.0 ± 0.4	−1.4 ± 1.3	−1.0 ± 0.7
		0.7 ± 0.7									
Prochlorococcus marinus MED4	Diel (14/10)	NH_4^+	n/a			−5.1 ± 1.7		−7.1 ± 0.9		−2.0 ± 1.9	
		4.0 ± 0.4									
Phormidium YNP Isolate	Diel	NO_3^-	n/a			1.9 ± 1.2		−7.5 ± 2.3		−9.4 ± 2.6	
		3.2 ± 0.9									
Rhodopseudomonas palustris	Continuous	NH_4^+	4			−22.4 ± 2.5		−13.1 ± 0.8		9.3 ± 2.6	
			6			−21.6 ± 1.4		−11.5 ± 1.0		10.1 ± 1.7	
			7			−28.6 ± 1.9		−12.9 ± 1.0		15.7 ± 2.1	
Chlamydomonas reinhardtii	Continuous	NH_4^+	4–6	11.7 ± 1.4	0.35	−11.7 ± 0.8		−6.1 ± 0.2		5.6 ± 0.9	
		−3.5 ± 0.6	8	29.9 ± 4.6	0.16	−9.7 ± 0.4		−4.7 ± 0.2		4.9 ± 0.5	
			10	28.9 ± 2.8	0.07	−8.6 ± 0.5		−2.2 ± 0.5		6.4 ± 0.7	
			12	32.7 ± 1.2	−0.02	−7.7 ± 0.4		−0.6 ± 0.1		7.1 ± 0.5	
			14	–	–	−6.6 ± 0.5		0.1 ± 0.3		6.7 ± 0.6	
			18	–	–	−5.5 ± 0.6		3.2 ± 0.3		8.7 ± 0.6	
	Diel (12/12)	NH_4^+	n/a	3.7 ± 0.5	0.18	−8.7 ± 4.5	−7.0 ± 2.1	−2.1 ± 1.4	−2.3 ± 0.5	6.6 ± 4.7	4.7 ± 2.2
		−1.3 ± 0.6	n/a	5.0 ± 0.4	0.29	−7.0 ± 1.1	−6.2 ± 1.1	−0.7 ± 0.4	−0.6 ± 0.6	6.3 ± 1.1	5.5 ± 1.3
			n/a	16.3 ± 0.2	0.01	−5.8 ± 0.3	−5.9 ± 1.0	2.4 ± 0.2	2.3 ± 0.0	8.2 ± 0.4	8.3 ± 1.0
Porphyridium cruentum	Diel (13/11)	NO_3^-				−15.3 ± 0.9		−8.5 ± 0.7		6.8 ± 1.1	

follows the same pathway as chlorophyll biosynthesis in eukaryotic algae (O'Brian and Thöny-Meyer, 2002).

R. palustris, a purple nonsulfur Alphaproteobacterium, was grown aerobically on NH_4^+ and under continuous light. This species is characterized by larger ε_{por} values than those seen either for algae or for purple sulfur bacteria. Bacteriochlorophyll was offset from biomass by an average of $11.7 \pm 3.8‰$ in a batch culture of *R. palustris* (Table 1), with ε_{por} values increasing from $9.3 \pm 2.6‰$ to $15.7 \pm 2.1‰$ over the course of a growth cycle ($n = 3$) (Fig. 2; Figure EA-1-1).

A value of ε_{por} of ~12‰ is significantly different from a typical ε_{por} value of 5–7‰. This probably results from use of a different initial precursor for bacteriochlorophyll synthesis in Alphaproteobacteria. In all organisms, the first committed precursor to chlorophyll and heme biosynthesis is δ-aminolevulinic acid (δ-ALA), which can be made from two different amino acid precursors. All cyanobacteria and algae convert the amino acid glutamate to δ-ALA. However, non-photosynthetic eukaryotes and Alphaproteobacteria (including *R. palustris*) use the Shemin pathway to synthesize δ-ALA from glycine (Beale and Weinstein, 1989; Beale, 2006). Glycine is derived from the amino acid serine, which is formed in a three-step process that involves a transfer of an amino group from glutamate. Thus, even though the ultimate source of N for both pathways is glutamate, the additional steps involved in conversion of glutamate to glycine yield glycine that is more ^{15}N-depleted than glutamate. A previous report for *A. cylindrica* grown on different nitrogen sources showed that glycine is 2–4‰ more depleted in ^{15}N than glutamate (Macko et al., 1987). Similarly, McClelland and Montoya (2002) measured $\delta^{15}N$ values of amino acids from the cultured green alga *Tetraselmis suecica* and found a 4.3‰ offset. McCarthy et al. (2007) suggest that the offset may be even greater: they measured 9.2‰ depletion in the $\delta^{15}N$ value of glycine compared to glutamate from environmental samples of a diatom mat dominated by *Rhizosolenia*. Similarly, Chikaraishi et al. (2009) report an average difference between glutamate and glycine of 5.5‰ for cyanobacteria, 4.5‰ for green algae, and of 8.9‰ for red and brown algae. Together these results suggest glycine is systematically (by $5 \pm 3‰$) depleted in ^{15}N relative to glutamate.

Isotopic depletion in the glycine utilized by the Shemin pathway explains our data, as well as prior reports. Beaumont et al. (2000) found that ε_{por} values for the Alphaproteobacterium *R. capsulatus* grown on N_2 and NH_4^+ averaged 7.5‰ and 9.7‰ lighter than biomass, respectively. The relatively negative value of $\delta^{15}N$ in chloropigments of Alphaproteobacteria thus reflects an established property of glycine. This effect need not be specific to Alphaproteobacteria, but rather, to date it is only the Alphaproteobacteria that are believed to use glycine to make tetrapyrrole pigments. The overall range of observations suggests that the conventional (algal) chlorophyll biosynthetic pathway has a nitrogen isotopic fractionation of ~5–7‰, but the Shemin pathway has a fractionation of ~8–12‰, for a difference of approximately 3–5‰, *i.e.*, identical to the isotopic difference between glutamate and glycine.

3.3. Fractionations associated with chlorophyll synthesis in cyanobacteria

3.3.1. Freshwater cyanobacteria

Most surveys of the nitrogen isotope fractionation associated with chlorophyll biosynthesis have focused on eukaryotic phytoplankton. Here we focused most of our efforts on phylogenetically and environmentally diverse cyanobacteria, grown under different nutrient and light conditions. Chl-based growth profiles and isotope data were obtained for the freshwater species *Nostoc* sp. PCC 7120, *A. variabilis* ATCC 29413, and *Synechocystis* sp. PCC 6803 under multiple growth conditions; and for a mat-forming *Phormidium* sp. in a single experiment. Cultures were grown either under continuous or diel (12:12) light, and with different nitrogen sources. *Synechocystis* does not fix nitrogen, so it was grown on NH_4^+ and NO_3^-, whereas the diazotrophic *Anabaena* and *Nostoc* spp. were grown on NH_4^+, NO_3^-, and N_2, and *Phormidium* was grown on NO_3^- only. We measured the (initial) media $\delta^{15}N$ values (Table 1), without accounting for enrichment due to Rayleigh fractionation during nutrient consumption (Hoch et al., 1992), and then measured the $\delta^{15}N$ values for chlorophyll and biomass throughout the experiments (Table 1, Fig. 3). Figures EA-1-1–3 show growth rates and sampling time points.

The most striking feature of the results for fresh water species (Table 1, Fig. 3) is that they all have values of ε_{por} that are opposite in sign from fractionations seen in eukaryotic algae and anoxygenic photosynthetic bacteria. For species grown in continuous light, chlorophyll $\delta^{15}N$ values were on average ~10‰ more ^{15}N-enriched than biomass ($\varepsilon_{por} = -9.9 \pm 2.8‰$). These results are consistent with the data obtained by Beaumont et al. (2000) for *Anabaena* sp.

Since cyanobacteria have light-dependent circadian rhythms (Kondo et al., 1993), we also tested the effects of diel illumination on the fractionation associated with chlorophyll biosynthesis. The continuous-light experiments for *Nostoc* sp. and *Synechococystis* sp. using NO_3^- as N source were repeated on a 12:12 cycle (Figure EA-1-2). Separately, a freshwater mat-forming cyanobacterium (*Phormidium* sp.) that was initially isolated from a hotspring at Yellowstone National Park (Bosak et al., 2009) was grown on a 13:11 (L:D) cycle. For all of these cultures, the observed fractionation was similar to the continuous-light cultures (Table 1; Fig. 3). *Nostoc*, *Synechocystis*, and *Phormidium* isolates had values of ε_{por} ranging from −8.9 to −15.1‰ and averaging $-11.4 \pm 2.0‰$; this is within 1σ of the average obtained for continuous illumination.

However, on an individual species basis, there were small differences in ε_{por} values observed for the freshwater cyanobacteria species analyzed under a diel regime. Values of ε_{por} are slightly more negative (*i.e.*, absolute values of $\delta^{15}N_{chl}$ are more positive) in these cultures when grown in diel conditions and sampled both in the light and in the dark ($p < 0.02$ for *Nostoc* and $p < 0.002$ for *Synechocytis*). Values of ε_{por} for *Synechocystis* grown on NO_3^- average $-9.4 \pm 1.1‰$ under continuous light and $-13.0 \pm 1.1‰$ under diel light conditions. In *Nostoc* grown on NO_3^-, ε_{por} values average $-6.7 \pm 1.5‰$ under continuous light, and

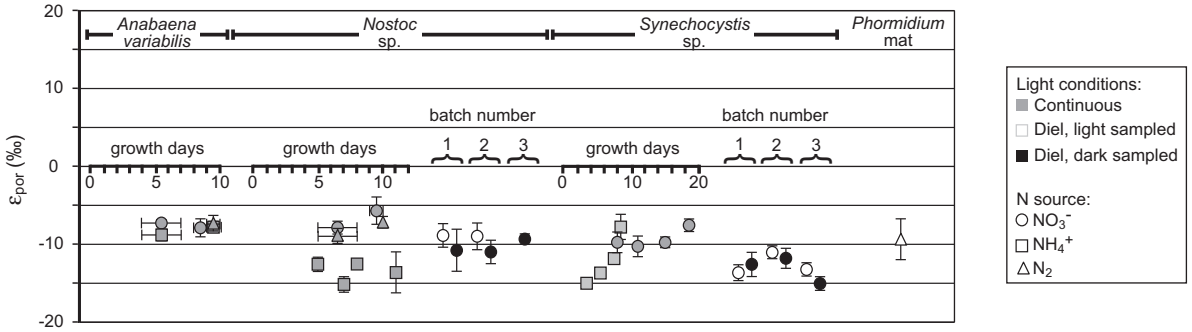

Fig. 3. Measured values of ε_{por} values for freshwater cyanobacteria. Shapes indicate N species supplied for growth, and shading distinguishes light regime. The growth days of the cultures at the time of sampling are shown on the *x*-axis for continuous light samples; else the designation (light or dark) and batch numbers are shown for diel samples. Growth data are shown in Figures EA-1-1 and EA-1-2, and ε_{por} as a function of growth rate is shown in Figure EA-1-1–3.

$-9.8 \pm 1.6‰$ under diel light (Table 1); *i.e.*, in both cases the enrichment of ^{15}N into chlorophyll is more strongly expressed under diel conditions. Isotopic differences between samples collected during light periods and their corresponding dark periods are not statistically significant. Since offsets between $\delta^{15}N_{cell}$ and initial media $\delta^{15}N$ values indicate that these cultures were not N limited, the differences in ε_{por} values between cultures grown under continuous and diel light conditions may result from differential cellular allocation of nitrogen resources as a result of variable enzymatic expression (Saito et al., 2011). However, in all cases, the magnitude and direction of ε_{por} is observed regardless of N substrate, growth rate, or illumination (Fig. 3). It appears to be universally true that the chlorophyll of freshwater cyanobacteria is enriched in ^{15}N.

The lack of an observed substrate effect is not surprising. When nitrogen is assimilated into cells, it is converted to NH_3, no matter what its starting oxidation state (NO_3^-, NH_4^+ or N_2). This NH_3 is then used to make the intracellular amino acid pools from which both cellular proteins and chloropigments are synthesized. Enzymes that convert N substrates to cellular NH_3, such as nitrate reductase and nitrogenase, have different fractionations (e.g., Hoering and Ford, 1960; Delwiche and Steyn, 1970; Waser et al., 1998; Granger et al., 2004). These fractionations should only affect values of $\delta^{15}N_{biomass}$ (or $\delta^{15}N$ of total amino acids) relative to $\delta^{15}N_{substrate}$; they should not affect $\delta^{15}N_{chlorophyll}$ relative to $\delta^{15}N_{biomass}$. Our data for cyanobacteria grown on NO_3^- and N_2 are entirely consistent with this idea.

In practice, however, the observed values of ε_{por} for *Nostoc* and *Synechocystis* grown on NH_4^+ (continuous light experiments) do show small substrate-dependent effects. We believe that this is an artifact of batch culturing. Values of ε_{por} of $-13.5 \pm 1.2‰$ and $-14.0 \pm 1.2‰$, respectively, are significantly outside the window of $-6‰$ to $-9‰$ observed for other media under continuous light ($p < 0.002$; Table 1). It is possible that these extreme values may result from nitrogen recycling under nitrogen stress, as the NH_4^+ concentration in these cultures was estimated to be limiting over the duration of the experiments. By the end of the experiments, *Nostoc* and *Synechocystis* both showed biomass values of $\delta^{15}N$ that were more enriched than $\delta^{15}N_{NH4+}$ of the starting media, suggesting that enough NH_4^+ is consumed to induce ^{15}N-enrichment of the remaining NH_4^+ by Rayleigh fractionation. In contrast, the final *Anabaena* biomass was more ^{15}N-depleted than the starting value of $\delta^{15}N_{NH4+}$, suggesting it did not become N-limited. Consequently, its value of ε_{por} was not affected.

Nitrogen limitation may increase the magnitude of ε_{por} values through a difference in the synthesis and recycling rates of chlorophyll and bulk biomass. As biomass accumulates, it should become ^{15}N-enriched through Rayleigh fractionation until it approaches the starting reactant $\delta^{15}N$ value of the N substrate. However, since the final values of $\delta^{15}N_{biomass}$ for *Nostoc* and *Synechocystis* were more positive than the starting NH_4^+, biomass must be recycling into a ^{15}N-depleted dissolved form that is not bioavailable. This is required in order to maintain isotope balance. Therefore the measured values of $\delta^{15}N_{biomass}$ that are heavier than the initial $\delta^{15}N_{NH4+}$ of the medium must represent time-averaged product that is biased toward the later days of the culture. If the chlorophyll component has a shorter residence time than bulk biomass – *i.e.*, it is newly synthesized from glutamate at a higher rate – the ^{15}N content of new chlorophyll will reflect an even more highly Rayleigh-fractionated substrate. This will cause the apparent fractionation between chlorophyll and biomass to increase beyond the enzymatic fractionation associated with (instantaneous) chlorophyll biosynthesis. Such Rayleigh distillation effects on ε_{por} are only possible in closed systems and may not be significant for most environmental samples.

Alternatively, some component of any observed differences in ε_{por} may be due to variability in the intracellular ^{15}N fractionation between glutamate and bulk biomass ($\varepsilon_{glu} = \delta^{15}N_{biomass} - \delta^{15}N_{glu}$). Glutamate is a central intermediate in the formation of other amino acids that are used to build the bulk of cellular protein. The normal kinetic isotope effects associated with these reactions cause ^{15}N-depletion in the downstream amino acids, with residual ^{15}N accumulation in glutamate. Changes in ε_{glu} may then result from changes in cellular N partitioning. Macko et al. (1987) found total protein to be $3.5 \pm 0.3‰$ enriched in ^{15}N compared to total biomass in six species of algae, and glutamate to be $\sim 3.3 \pm 0.9‰$ more enriched in ^{15}N than whole

protein. More recently, Chikaraishi et al. (2009) measured $\delta^{15}N$ values of amino acids and biomass for a suite of different phytoplankton species, finding ε_{glu} values from −2.9‰ (the cyanobacterium *A. cylindrica*) to +2.5‰ (the green alga *Chlorella* sp.). These ranges could help explain some variability in ε_{por} values.

To summarize, the variability in expressed values of ε_{por} within cyanobacterial species is affected minimally or not at all by the choice of N substrate (N_2, NO_3^-, or NH_4^+). Expressed values of ε_{por} are affected modestly by growth under different light regimes, by the degree of N-limitation experienced under cellular growth conditions, or by differences in the intracellular partitioning of ^{15}N among amino acids. However, none of these factors are large enough to affect the gross observation: values of ε_{por} are always negative for freshwater cyanobacteria. These observations from cultures are supported by the only *in situ* value of cyanobacterial ε_{por} measured to date – a freshwater lake in Japan in which ε_{por} was determined to be −13‰ to −16‰ (Katase and Wada, 1990). These values indicate potentially even stronger enrichment of ^{15}N in the chlorophyll of cyanobacteria growing naturally in freshwater systems.

3.3.2. Marine cyanobacteria

To compare differences across environmental regimes, as well as to broaden the taxonomic representation among our experiments, we also examined three species of marine cyanobacteria. Growth profiles and isotope data were obtained for marine *Synechococcus* sp. WH8102 under multiple conditions, including growth on NO_3^- and NH_4^+, and in continuous light and diel (12:12) cycles. We also tested two additional environmentally-representative taxa under diel (14:10) conditions. These were the unicellular, marine nitrogen-fixing species *C. watsonii* (grown on N_2) and the marine prochlorophyte *P. marinus* MED4 (grown on NH_4^+). Figures EA-1-1–3 show growth rates and sampling time points, and isotope data are given in Table 1.

The data again show that choice of substrate is the least significant determinant of values of ε_{por} (Fig. 4), and that nutrient recycling or intracellular partitioning have small but measurable effects. Across all of the marine species data, the average expressed value of $\varepsilon_{por} = -0.9 \pm 1.3$‰; while by N substrate, NO_3^- $\varepsilon_{por} = 1.4 \pm 1.3$‰, NH_4^+ $\varepsilon_{por} = -1.5 \pm 1.9$‰, and N_2 $\varepsilon_{por} = -1.2 \pm 1.0$‰. The remarkable feature of these marine cyanobacterial data is that in strong contrast to freshwater strains, values of ε_{por} now are clustered around 0‰, *i.e.*, effectively no fractionation of ^{15}N between chlorophyll and biomass.

By species, the ε_{por} data for *C. watsonii* (order Chroococcales) reflect diel growth, sampled in both the light (-1.4 ± 1.3‰) and dark (-1.0 ± 0.7‰) at late log phase. These values are not significantly different from *P. marinus* (order Prochlorales), also grown on a diel cycle and sampled a single time point (-2.0 ± 1.9‰). All three results are very similar, despite the different taxonomic affiliations and growth substrates (N_2 vs. NH_4^+) for these species.

Synechococcus was grown under both continuous light and diel conditions. When grown using NH_4^+, it had a value of ε_{por} averaging -0.9 ± 1.9‰ (continuous light). This is again the same as the values found for *Crocosphaera* and *Prochlorococcus*. When grown specifically using NO_3^-, *Synechococcus* had a value of ε_{por} averaging -0.5 ± 0.8‰ under continuous light, but a value of $+3.3 \pm 2.8$‰ under diel conditions, *i.e.*, chlorophyll became more ^{15}N-depleted when grown on diel rather than continuous light. The latter value is significantly different from the other results for *Synechococcus* (Table 1) and is approaching the values seen for eukaryotic algae. This effect was observed to be a function of time in culture (Figure EA-1-2). The same effect is not seen in other cyanobacteria, nor is it seen outside of the cyanobacteria: *Chlamydomonas* had the same ε_{por} value under continuous and diel light conditions (Table 1), although the magnitude of the fractionation again did increase with time in culture, probably due to NH_4^+ recycling (see Section 3.1).

Our data for *Synechococcus* sp. WH8102 differ from the previously published measurement of ε_{por} for *Synechococcus* sp. WH7803 from Sachs et al. (1999). In contrast to the small fractionations that we measured, Sachs et al. measured a value of ε_{por} of 10.1‰ for the offset between chlorophyll and *Synechococcus* biomass, *i.e.*, chlorophyll 10‰ more depleted in ^{15}N. Since this value differs dramatically from our data, not just for *Synechococcus* sp. but for all of the marine cyanobacteria we tested, we find this earlier

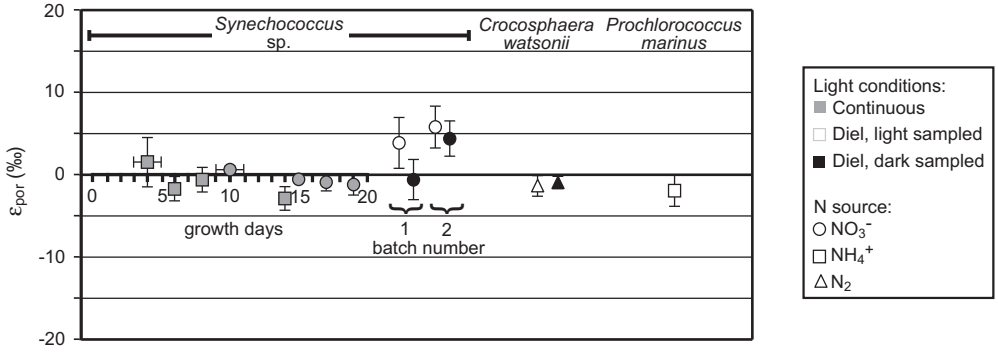

Fig. 4. Measured values of ε_{por} values for marine cyanobacteria. Shapes indicate N species supplied for growth, and shading distinguishes light regime. The growth days of the cultures at the time of sampling are shown on the *x*-axis for continuous light samples; else the designation (light or dark) and batch numbers are shown for diel samples. Growth data are shown in Figures EA-1-1 and EA-1-2, and ε_{por} as a function of growth rate is shown in Figure EA-1-1–3.

value irreproducible. It is possible that this earlier value is an artifact of culturing conditions (growth data were not presented), sample contamination, N-recycling in batch medium, or some other unexpected effect. When considered along with all of our other data for marine cyanobacteria, or just among our data for *Synechococcus*, the Sachs et al. (1999) value is a statistical outlier (Grubbs' test, $p < 0.05$). This is true even considering the time-evolving values of ε_{por} in our diel cultures. We suspect that the value reported by Sachs et al. (1999) is not representative either of *Synechococcus* spp. or of marine cyanobacteria in general. Analysis of environmental samples will be important to answer this question.

The overall pattern of results suggests that marine and freshwater species occupy two distinct categories of ^{15}N fractionation among cyanobacteria. The distinction between freshwater ($\varepsilon_{por} \sim -10‰$) and marine ($\varepsilon_{por} \sim 0‰$) chlorophyll ^{15}N fractionation is not sorted phylogenetically. The marine strains tested include representatives of the orders Prochlorales and Chroococcales, while the freshwater strains tested include the orders Chroococcales, Nostocales, and Oscillatoriales. The entire data set for cyanobacterial ε_{por} values (50 data points) encompasses cultures that (i) were grown with a variety of N substrates; (ii) were measured at growth time-points that included early-, mid-, and late-log phases as well as stationary phase; (iii) were grown on a variety of light regimes; and (iv) represent a wide diversity of cyanobacterial taxa. There are no discernable patterns in the outcome other than the growth regime: freshwater or marine. Taxonomic variability of results within these two categories is not statistically significant. We therefore believe that the explanation for these categories lies within a differing physiology of N utilization that is a function of growth medium (salinity, pH, ionic strength, metal availability, or some other factor (e.g., Zerkle et al., 2008)).

3.4. Implications of ε_{por} values for the interpretation of sedimentary isotope data

These results have several implications for the use of chlorin and porphyrin N isotopes in paleoceanography. In order for values of $\delta^{15}N$ of chloropigments to be a useful proxy for past nutrient conditions, the value of ε_{por} must be well constrained. To date, chlorin and porphyrin $\delta^{15}N$ data have been uniformly interpreted assuming a 5‰ value for ε_{por} (Sachs and Repeta, 1999; Ohkouchi et al., 2006; Kashiyama et al., 2008a,b, 2010; Higgins et al., 2010). This assumption appears to be valid with regard to changing environmental conditions: in all phyla studied previously, as well as all taxa investigated in this work, the type of N substrate (N_2, NO_3^-, or NH_4^+) has little effect on chlorophyll ^{15}N fractionation. This suggests that values of ε_{por} would not be systematically affected by different redox conditions. In the absence of differences in species composition, the proportions of N_2, NH_4^+ and NO_3^- utilized by the marine phytoplanktonic community would not affect the observed magnitude of ε_{por}, even if such changes in nutrients affected the bulk values of sedimentary $\delta^{15}N$. In other words, values of $\delta^{15}N$ of total sedimentary nitrogen and of chloropigments always would be expected to change in parallel. This would be the predicted pattern in a sedimentary environment in which all export production is dominated by sinking biomass of eukaryotic phytoplankton (or eukaryotes plus purple sulfur bacteria).

However, phylum-specific differences do have the potential to exert a strong effect on the interpretation of ε_{por} values. Environmental samples from extremely ferruginous oceans or lacustrine systems could contain significant quantities of porphyrins derived from bacteriochlorophylls of Alphaproteobacteria. Such environments may have occurred frequently in the latest Archean (Kappler et al., 2005) and possibly in the Neoproterozoic (Canfield et al., 2007; Johnston et al., 2010). These organisms may have alternated with sulfide-oxidizing photoautotrophs, especially in the Paleo- and Mesoproterozoic (e.g., Canfield, 1998; Brocks et al., 2005; Lyons et al., 2009), regardless, throughout the majority of the Proterozoic, export production of prokaryotic biomass was likely to have been more significant than eukaryotic biomass (Logan et al., 1995; Close et al., 2011). Because the magnitude of ε_{por} for anoxygenic photosynthetic Alphaproteobacteria (~8–12‰) is nearly twice that for sulfide oxidizers or oxygenic eukaryotes (~5–7‰), signatures of Alphaproteobacteria would be completely distinct from the ε_{por} signatures of other taxonomic groups of primary producers.

Thus, if thermally-immature Precambrian rocks could be analyzed for porphyrin $\delta^{15}N$ values, and bulk $\delta^{15}N$ values of kerogen-bound nitrogen represented unaltered, primary biomass from surface waters, compound-specific porphyrin analyses could help elucidate which photosynthetic organisms dominated export production in Precambrian oceans. Such data may be especially informative for rocks deposited during the Archean and Paleoproterozoic, before the rise of eukaryotic phytoplankton. Indeed, it is possible to suggest that in general, values of ε_{por} recorded in sediments would follow a pattern loosely stratified by Eon: very positive in the Archean (anoxygenic photosynthesizers), near zero in the Proterozoic (fewer anoxygenic photosynthesizers, and general dominance of cyanobacteria), and moderately positive to zero in the Phanerozoic, depending on the fraction of eukaryotic plankton.

In marine environments dominated by cyanobacteria, ε_{por} values would shift towards being within $0 \pm 2‰$ of the values of $\delta^{15}N$ for total sedimentary nitrogen. However, in the event that a cyanobacterial signal in sediments was influenced strongly by freshwater species, perhaps resulting from a "lens" of stratification, it is possible that values of ε_{por} recorded in sediments could be negative. Such situations may have occurred during the Eocene, when freshwater ferns (*Azolla* spp.) were present in the Arctic (Speelman et al., 2009), or in the Holocene Black Sea (van der Meer et al., 2008). In earlier work by our group, we measured ε_{por} values in Pleistocene Mediterranean sapropels, for which very modest salinity changes have been proposed (van der Meer et al., 2007). At the time, we interpreted our results (based on absolute values of $\delta^{15}N$ of total sedimentary N near 0‰) as indicating a relatively large contribution of cyanobacterial N_2-fixation to export production (Higgins et al., 2010). We now suggest that this

interpretation was incorrect: although they decreased slightly in sapropel horizons, values of ε_{por} remained near 5‰ both within sapropel horizons and between sapropel events. We did not at the time know of the characteristic ε_{por} signatures for cyanobacteria, but based on these values of ε_{por}, the sapropel results are not consistent either with dominant export of marine or of freshwater cyanobacteria. To date only one lacustrine example has been studied; but it did have a very negative value of ε_{por} (Katase and Wada, 1990). This suggests that – if present – signals for freshwater cyanobacteria should be detectable. Further tests of this principle are necessary in a range of environmental systems.

In general, in the record of Phanerozoic marine sediments for which porphyrin isotope data exist, values of ε_{por} show relatively little variation. Near-zero and/or negative ε_{por} values have not yet been detected in any marine system. The pattern of "eukaryotic" ε_{por} values near 5‰ even appears to hold during Cretaceous Oceanic Anoxic Events (OAEs), when productivity has been suggested to have been influenced by a relatively large increase in cyanobacterial N_2-fixation. Other results to date include a sample of a Triassic oil shale (Chicarelli et al., 1993), two data points for the Bonarelli shale of Cretaceous OAE 2 (Ohkouchi et al., 2006), four additional samples from the Bonarelli and one from the older Selli level (Kashiyama et al., 2008a), and seven horizons from the middle Miocene (Kashiyama et al., 2008b). Most of these values are ~4‰ (rather than a typical ~5‰) depleted in ^{15}N relative to coeval sedimentary nitrogen or kerogen; with the exception of the Triassic sample, which has a smaller apparent ε_{por} value of around 2–3‰. Assuming no diagenetic alteration of bulk $\delta^{15}N$ signals, values of ε_{por} near 4‰ for OAEs represent approximately a 1‰ shift toward a marine cyanobacterial endmember of $\varepsilon_{por} = 0‰$. Such changes in ε_{por} values are thus consistent with a large relative change in the cyanobacterial population, but place a maximum constraint on the cyanobacterial fraction of total export flux at ~20%. The Triassic data suggest a larger cyanobacterial contribution of ~50% of the total organic burial. Overall, these data from the recent Phanerozoic suggest that cyanobacteria do not contribute a major component of preserved export production and/or that their chloropigments are not well preserved. Such a conclusion is consistent with the idea that in the modern ocean, eukaryotic phytoplankton are responsible for most of the export flux out of surface waters, even in basins that may have a large population of N_2-fixing cyanobacteria contributing to their surface N pools (e.g., Altabet, 1988).

4. CONCLUSIONS

Measurements of the ^{15}N fractionation between chlorophyll and biomass in a taxonomically diverse group of photosynthetic organisms suggest that fractionation factors fall into four groups. Eukaryotic algae and most anoxygenic photosynthetic bacteria produce chloropigments that are 5–7‰ more ^{15}N-depleted than biomass, *i.e.*, ε_{por} values of ~5–7‰. Purple nonsulfur Alphaproteobacteria are characterized by greater fractionation (ε_{por} of ~8–12‰) due to a different amino acid precursor for bacteriochlorophyll. Cyanobacterial ε_{por} values fall into two groups. Freshwater cyanobacteria are characterized by apparent inverse fractionations for chlorophyll biosynthesis, with ε_{por} values of ~−10‰ (enriched in ^{15}N relative to biomass). Marine cyanobacteria have effectively no fractionation, with ε_{por} values averaging ~0‰. Offsets between chlorins/porphyrins and bulk N in marine sedimentary sections studied thus far are around 4–5‰. This suggests that in the Mesozoic and Cenozoic, eukaryotes have dominated marine export production.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2011.04.024.

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