Silicon content of individual cells of *Synechococcus* from the North Atlantic Ocean

¹Daniel C. Ohnemus, ¹Sara Rauschenberg, ^{2,3}Jeffrey W. Krause, ⁴Mark A. Brzezinski, ⁵Jackie L. Collier, ⁵Sabrina Geraci-Yee, ⁶Stephen B. Baines, ¹Benjamin S. Twining

¹Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine, USA

²Dauphin Island Sea Lab, Dauphin Island, Alabama, USA

³Department of Marine Sciences, University of South Alabama, Alabama, USA

⁴Marine Science Institute and the Department of Ecology Evolution and Marine Biology, University of California, Santa Barbara, California, USA

⁵School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York, USA

⁶Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York, USA

keywords: silicon; Synechococcus; synchrotron x-ray fluorescence; SXRF; elemental quotas

Abstract

The widely distributed marine cyanobacterium *Synechococcus* is thought to exert an influence on the marine silicon (Si) cycle through its high cellular Si relative to organic content. There are few measurements of Si in natural populations of *Synechococcus*, however, and the degree to which *Synechococcus* from various oligotrophic field sites and depths accumulate the element is unknown. We used synchrotron x-ray fluorescence to measure Si quotas in individual *Synechococcus* cells collected during three cruises in the western North Atlantic Ocean in the summer and fall, focusing on cells from the surface mixed layer (SML; <10 m) and the deep chlorophyll maximum (DCM). Individual cell quotas varied widely, from 1 to 4700 amol Si cell⁻¹, though the middle 50% of quotas ranged between 17 and 119 amol Si cell⁻¹. Mean station-specific quotas exhibited an even narrower range of 31 – 72 amol Si cell⁻¹. No significant differences in Si quotas were observed across cruises or among stations, and no effect of ambient silicic acid concentration on quotas was observed within the narrow range of silicic acid concentrations encountered (0.6 – 1.3 μM). Despite this small range in ambient silicic acid, cells collected from the SML had an average of two-fold more Si than cells collected from the DCM. Differences in Si content with depth may be related to observed differences in the dominant *Synechococcus* clades between the SML and DCM habitats, determined by *petB* gene sequencing.

Introduction

Photoautotrophic picocyanobacteria from the genus *Synechococcus* are found in nearly all ocean surface waters and have been shown to accumulate Si. Surprisingly, they can attain biomass-normalized cellular Si:S and Si:P mole ratios that are, on average, up to 50% of those in siliceous diatoms (Baines et al. 2012). *Synechococcus* are most abundant in nutrient-rich upwelling systems and in coastal and estuarine environments (Partensky et al. 1999), but they are also found in oligotrophic open-ocean systems like the Costa Rica Dome at abundances as high as 10⁵ to 10⁶ mL⁻¹ (Saito et al., 2005; Stukel et al., 2013). Persistent accumulation of Si by *Synechococcus* presents an additional pathway for Si uptake that is not controlled by diatoms. Furthermore, recent analyses have suggested that ≥ 75% of the euphotic zone biogenic silica (bSi) pool is “detrital” (i.e. not associated with living diatoms) (Krause et al., 2010; Marquez, 2015). Contributions by *Synechococcus* would have been systematically included in this detrital pool instead of within picoplankton size cells. Therefore, the direct consideration and inclusion of *Synechococcus* biomass may be important to accurately modelling the Si cycle in ocean regions where *Synechococcus* biomass is significantly higher than diatoms.

Since the discovery of Si accumulation by marine *Synechococcus* by Baines et al. (2012), other studies have investigated this organism’s potentially outsized effects on other elemental cycles and on vertical C export. Tang et al. (2014) demonstrated accumulation of Mg and Si in the protein- and carbohydrate-rich extracellular polymeric substances (EPS) of degrading *Synechococcus* cultures and in water column micro-aggregates near Bermuda. Deng et al. (Deng et al., 2015b) further showed that *Synechococcus* detritus scavenges C from exopolymers and dissolved

organic matter, forming rapidly sinking ($440\text{--}660\text{ m d}^{-1}$) aggregates that may export 2 – 3 times more C than in *Synechococcus* cells alone. Recent results from the *Tara* oceans expedition have implicated *Synechococcus* and its phages as having central, strategic roles in C export networks of the oligotrophic gyres (Guidi et al., 2016). Scavenging and vertical export by *Synechococcus* biomass thus appears to be more significant to a range of elemental budgets than previously thought (Lomas et al., 2010).

The *Synechococcus* genus has wide temperature, nutrient, and light tolerances due to its considerable genetic diversity. Numerous clades have been defined using genetic techniques, and many clades show preferences for specific marine niches (Tai, 2009) though little is known about the degree to which natural field populations accumulate Si. With the abundance and geographic range of *Synechococcus* expected to grow in response to global climate change (Flombaum et al., 2013), their influence on the marine Si cycle and other elemental cycles may also grow. The observed expansion of the oligotrophic gyres (Polovina et al., 2008) and decadal declines in open-ocean diatom biomass (Krause et al., 2009) underscore the importance of understanding the interactions of natural field *Synechococcus* populations with Si. How do typical *Synechococcus* cellular Si quotas (i.e. mol Si per cell) vary across environments, seasons, and within various marine niches (e.g. surface mixed layer [SML], deep chlorophyll maximum [DCM])? We investigated these questions through synchrotron x-ray fluorescence (SXRF)-based examination of single-cell elemental quotas in field-collected *Synechococcus* during the summer and early fall in the western North Atlantic.

Methods

Bottle sampling: BATS and BVAL cruises

Whole seawater samples were collected during Bermuda Atlantic Time Series (BATS) cruises in July, 2012 (BATS cruise 283) and October, 2012 (BATS 286) and during a BATS validation cruise in September-October, 2013 (BVAL 48; stn. 2) on the *R/V Atlantic Explorer* [Fig.1]. The BVAL cruise then continued south towards Puerto Rico, occupying several stations from which only SML samples were analyzed. Seawater was collected from the SML (5 – 10 m sampling depth) and the depth of the subsurface DCM (75 – 88m sampling depth; determined for each cast via *in situ* fluorometry) using the ship's CTD rosette. All reported parameters were measured on water collected from the same bottles. For single-cell SXRF mapping, whole seawater was preserved with glutaraldehyde at a final concentration of 0.25% and cells were deposited on Au electron microscopy grids using centrifugation at 1700 rcf for 30 minutes. Grids were briefly rinsed with deionized water to remove residual salt. Microscopy was used to locate orange-fluorescing, single *Synechococcus* targets for later SXRF analyses using an excitation wavelength of 480 nm and a long-pass emission filter. Cell size (c.a. 1 μm) and visual morphology (ovoid shape) were also used to distinguish *Synechococcus* cells from other auto-fluorescing cell types.

A wide range of values were observed for several environmental parameters in part due to local sampling site variability within cruises [Table 2]. This variation is likely due to a combination of vessel drift and advection of eddy features past the ship's position. Samples analyzed from BATS cruise 283 (July 2012) were collected during two casts on sequential days. *Synechococcus* abundances and small bSi (0.4 – 3 μm size-fraction) were notably higher at the SML than at the DCM on cast 1, while cast 3 exhibited similar values for both parameters at both depths. SXRF samples from BATS cruise 286 (Oct 2012) were also collected during two casts on sequential days, with significantly higher DCM *Synechococcus* abundances on cast 3. During the BVAL cruise, *Synechococcus* cell abundances in the SML generally increased towards the south, though compared to the BATS cruises, *Synechococcus* cell abundances and bSi were both generally lower and exhibited smaller ranges during this cruise.

SXRF methodology

Samples were analyzed at the Advanced Photon Source beamline 2-ID-E with an incident energy of 10 keV using protocols similar to those previously described (Twining et al., 2011; 2003). Focused beam size was up to $\sim 0.4 \mu\text{m}$ in the vertical and $\sim 0.8 \mu\text{m}$ in the horizontal. Step sizes of $0.1 \mu\text{m}$ were used to over-sample target cells and background areas. The pixels covering the cell were averaged, and the mean signal from neighboring cell-free areas was subtracted from this average. The background-corrected concentration was then multiplied by the area of the analyzed cell region, resulting in a mol cell^{-1} measurement referred to herein as the cell quota.

Four detector elements collected SXRF spectra simultaneously at each pixel, and fluorescence data from each detector was quantified independently using NBS Standard 1833 and an AXO thin-film SXRF standard. Lighter elements (Si, P) that are absent in the standards were fit using a least squares linear regression through the $\log_{10}$ of expected quantum yield for the elements present in the standards, as detailed in Núñez-Milland et al. (2010).

For the picocyanobacteria analyzed in this study, slight differences in geometric orientation of the detector elements relative to the sample, combined with the shallow 15° observation angle to the sample grid (which has vertical topography significantly larger than many cells) occasionally created inconsistencies in the concentrations measured by the four detector elements. Detectors generally agreed on quantifications of high atomic mass (Z) elements (e.g. Fe, Ni), but occasionally two or three detectors would measure anomalously low quantifications for the low-Z elements (Si, P, S). We suspect that in these cases, fluorescent x-rays emitted by target cells were being shaded by the Au grid bars or by larger nearby cells, with the effect more noticeable for low-Z elements which emit lower-energy fluorescent x-rays that are more prone to absorption. This effect was detector-specific, implicating the geometry of the sample grid, cell region of interest, and detector position. To locate and compensate for this effect, for each mapped region of interest (ROI) and detector we plotted the measured elemental quotas (normalized to the highest detector) versus Z using MATLAB (The Mathworks Inc., Natick, MA, USA). Shaded

detectors were identified as those that exhibited a roughly linear decrease in relative quantification at lower Z. Data from shaded detectors were excluded in the final elemental analyses and from detection limit and sensitivity determinations.

SXRF uncertainty calculations in lieu of detection limits

Element-specific background levels vary from cell to cell depending on the target and its location on each grid, so that detection limits varied among runs, grids, and locations on grids. To estimate detection limits, we quantified cell ROI and background (non-cell) ROI uncertainties for each scan using the 1-sigma fitting uncertainties reported by the beamline MAPS software during elemental peak-fitting of the SXRF energy spectra (Vogt, 2003). We report the median target cell ROI uncertainty ± 1 s.d., and median target cell/background ratios in Table 1. For Si and S, cell ROI measurements have similar uncertainties (typically less than 5%). For P, cell ROIs have uncertainties that are typically 10-17%. On average, fluorescence signals in cell ROIs were 148% (Si), 631% (P) and 394% (S) of the signals in background ROIs, which themselves could be quantified with uncertainties of less than 10%.

Statistical Analyses

Generalized linear modeling was performed using the software JMP®, Version 10, SAS Institute Inc., Cary, NC. We tested the significance of cruise, station (nested within cruise, indicated by [Cruise] in Table 3) and sampling depth (i.e. SML or DCM) as fixed, independent effects on the dependent variables of log₁₀-transformed cellular Si, P, and S quotas as well as Si:P, Si:S, and P:S quota ratios. Effects with an associated p-value of < 0.05 were treated as significant and are discussed further in the text.

Bulk bSi quota measurements

In field samples, bulk biogenic silica concentration (bSi) was determined for two size fractions: >3 μm (here, “large bSi”) and 0.4 – 3.0 μm (“small bSi”) collected by sequentially filtering 2.5 to 3.0 L of seawater through 3.0 μm and then 0.4 μm polycarbonate filters (47 mm diameter). Filters were folded into quarters, placed in cryogenic vials, dried at 60° C, and sealed until analysis on shore in Teflon tubes. Both bSi and lithogenic silica were quantified from the same sample using the procedure of Brzezinski and Nelson (1995). Because some lithogenic silica can dissolve during NaOH digestion, we corrected bSi values by subtracting 15% of the measured lithogenic concentration, an empirically determined maximum correction factor for marine samples (Ragueneau and Tréguer 1994).

Flow cytometric enumeration of Synechococcus

Quantification of *Synechococcus* by flow cytometry followed the general procedure of Collier and Palenik (2003). Briefly, duplicate 1.5 mL subsamples of raw seawater were preserved as quickly as possible after collection at a final concentration of 0.25% EM-grade glutaraldehyde, incubated in the dark for 30 minutes at room temperature, and then frozen at -80°C until analysis on a four-color FACSCalibur (BD Biosciences, San Jose, CA, USA) flow cytometer for 10 to 15 minutes at a flow rate of

1 $\mu\text{L s}^{-1}$ with detectors set to an appropriate gain and a log scale. As an internal standard, 10,000 fluorescent beads (Polysciences Fluorescbrite PlainYG, 1.0 μm diameter) were added to 1 mL of each sample just before analysis. List-mode data files were imported into FlowJo (v6; Ashland, OR, USA) and groups of signals representing *Synechococcus* were identified and enumerated by their fluorescence and scatter characteristics.

Cyanobacterial community structure

Particles were collected from 1.5 to 2 liters of seawater in two size fractions as described for bulk Si, and total DNA was extracted from the 0.4- μm fraction using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) according to the manufacturer's instructions. Oligonucleotide primers modified from Mazard et al. (2012) were designed to amplify a 514 basepair region of all known marine *Synechococcus* and *Prochlorococcus* *petb* genes (encoding cytochrome *b₆*; petBf 5'-TACGAYTGGTTYCARGARMG and petBr 5'-AACGTRTGGRAGRCTRTARAA). For multiplexing of amplicon sequencing, ten forward and ten reverse primers, each with a distinct 16 base barcode (designed following the recommendations of Pacific Biosciences, Menlo Park, CA, USA), were synthesized by IDT (Coralville, Iowa, USA). Triplicate 20 μL PCR reactions were done for each sample using 1 to 3 μL of purified DNA (typically 2 ng), 10 μL of Phusion 2X Master Mix (NEB, Ipswich, MA, USA), and 0.5 μM of forward and reverse barcoded primers. The PCR conditions were initial denaturation at 94°C for 5 minutes, 30 cycles of 30 seconds at 94°C, 30 seconds at 40°C, and 45 seconds at 72°C, followed by a final extension at 72°C for 10 minutes and a 4°C hold. The replicate PCR products were mixed, gel-purified, then quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA). Equal amounts of PCR product from each of ten uniquely barcoded samples were pooled and submitted to the University of Delaware Sequencing & Genotyping Center (Newark, DE, USA) for sequencing on the Pacific Biosciences RSII Single-Molecule Sequencer using one SMRT Cell.

The raw data were processed using the SMRT Analysis software (Pacific Biosciences) with default settings. QIIME (1.9.1, implemented in Oracle VM VirtualBox 5.0.8; (Caporaso et al., 2010)) scripts were used to demultiplex the reads of insert, remove incomplete sequences lacking barcodes or primers at either end, and trim barcodes and primers from complete sequences. A reference alignment and phylogenetic tree constructed using all unique cyanobacterial *petB* sequences covering the full amplified region available in GenBank in November 2015 revealed that sequences were generally at least 90% identical within distinct clusters representing known clades of *Synechococcus*, and less than 90% identical between clusters. We therefore used the QIIME script pick_otus.py (Edgar, 2010) to place the sequences into operational taxonomic units (OTUs) at 90% sequence identity. OTUs with less than 0.01% of the total sequences were removed, and the most abundant sequence in each of the remaining OTUs was used, along with the reference alignment, for taxonomic assignment of each OTU based on sequence similarity (Caporaso et al., 2010; Wang and Dei, 2003) and to search for and remove potential

chimeric sequences with both ChimeraSlayer (Haas et al., 2011) and blast_fragments (Altschul et al., 1990).

The representative sequences for the resulting 164 OTUs were aligned with the reference *petB* set using ClustalW in BioEdit 7.2.5 (Hall, 1999). A phylogenetic tree (neighbor-joining, Tamura 3-parameter model with gamma=1 and unequal rates among lineages) was constructed in MEGA6 (Tamura et al., 2013) to confirm that each representative sequence grouped with one or more sequences from cultured isolates belonging to the clade, as identified by Mazard et al. (2012), to which its OTU had been assigned in QIIME (the most abundant being *Synechococcus* clade I, II, III, XVI, or WPC1). Two groups of OTUs were found in strongly supported but unnamed clusters, containing only environmental sequences from this and other studies (no cultivated strains); these are identified here as U1 and U2. Because of their low abundance, OTUs belonging to clades IV, V/VI/VII, or clustering with strain KORDI100 have been summed along with 4 taxonomically unassigned OTUs into 'other'. *Prochlorococcus* sequences, which are not the focus of this study, have been excluded from the data presented here but will be presented elsewhere.

Results

Single-cell quotas and quota ratios for Si, P and S

Individual *Synechococcus* cellular quotas for Si were highly variable, ranging from 1 – 4694 amol Si cell⁻¹ [Fig. 2]. However, more than 80% of samples fell within a more moderate range of 10 and 400 amol Si cell⁻¹. Median and geometric mean Si quotas were 44 and 47 amol Si cell⁻¹, respectively, with 25% and 75% quantiles of 17 and 119 amol Si cell⁻¹. Although cell-associated Si quotas were quite variable, cell Si SXRF peaks were indistinguishable from background abundances in only 3 of 118 *Synechococcus* cells analyzed: two at BVAL-2 DCM and one at BVAL-5 SML.

Generalized linear modeling [Table 3] found no significant effect of cruise or station on Si quotas [Table 3; $p > 0.05$]. Depth, however, was found to significantly affect Si quota, despite no major vertical variability in dissolved silicic acid concentrations (see below). Cells from the SML had c.a. two-fold more Si than cells from the DCM; least-square geometric mean quotas for SML and DCM cells were 67 and 31 amol Si cell⁻¹, respectively [Fig 2].

Quotas of cellular P and S—frequently used as proxies of cell biomass to compensate for natural variations in cell size, morphology, and macronutrient status (Twining et al., 2011)—were also measured using SXRF [Table 1; Fig. 2]. (C and N are too light to be directly measured with SXRF.) Both of these essential elements showed less variability than Si. Median and geometric mean P quotas were 22 and 25 amol P cell⁻¹, respectively, with 25% and 75% quantiles of 14 and 32 amol P cell⁻¹. Median and geometric mean S quotas were 47 and 55 amol S cell⁻¹, respectively, with 25% and 75% quantiles of 23 and 117 amol S cell⁻¹.

Generalized linear modeling [Table 3] showed that cruise, but not station or depth, significantly affected P quotas; least-square geometric mean P quotas were lower

during the July cruise ($18 \text{ amol P cell}^{-1}$) than the Sept/Oct cruises (BATS286: $28 \text{ amol P cell}^{-1}$; BVAL: $40 \text{ amol P cell}^{-1}$). All variables (cruise, station, and depth) were significant factors affecting S quotas; least-square geometric mean S quotas were higher during the July cruise ($70 \text{ amol S cell}^{-1}$) than the Sept/Oct cruises (both: $34 \text{ amol S cell}^{-1}$). Similar to Si, S quotas were higher in the SML ($72 \text{ amol S cell}^{-1}$) than the DCM ($26 \text{ amol S cell}^{-1}$).

Ratios of P:S varied significantly across cruises, stations, and depth, reflecting the underlying variability in the individual elements. Ratios of P:S were lower in the SML (0.30 mol P:mol S) than at the DCM (1.29 mol P:mol S) due to S elevation in the SML, while P:S ratios were lower during the July cruise (0.29 mol P:mol S) than the Sept/Oct cruises (0.73 and 1.12 mol P:mol S in BATS286 and BVAL, respectively) due to lower P values during the mid-summer. Ratios of Si:S did not significantly vary across cruise, station, or depth because Si and S varied in parallel with depth. Least-square geometric mean Si:P ratios were notably higher in the SML ($2.80 \text{ mol Si:mol P}$) than at the DCM ($0.63 \text{ mol Si:mol P}$), reflecting higher Si accumulation by SML cells.

Lack of relationships between Synechococcus Si content and major environmental parameters

Several independently measured environmental parameters, including bSi concentration, bSi size fractionation, and *Synechococcus* abundance, varied broadly across the three cruises. We did not observe significant relationships between these parameters and *Synechococcus* Si quotas [Fig. 3] (Pearson rho values $+0.16$ to $+0.34$; p-values all > 0.31). Ambient dissolved SiOH_4 concentrations also showed no relationship to *Synechococcus* cellular Si quotas (Pearson rho -0.22 ; p-value 0.52), although in accordance with the Sargasso Sea sampling region, only a small absolute range for this parameter ($0.6 - 1.3 \text{ } \mu\text{M}$) were encountered in upper water column *Synechococcus* habitats during the cruises.

Clade identity of SML and DCM Synechococcus communities

Synechococcus community assemblages within the SML and DCM were determined by *petB* (cytochrome b_6) gene sequences [Fig. 4]. *Synechococcus* communities from the SML were consistently dominated by OTUs representing clade III as identified by Mazard et al. (2012), with a lesser contribution of a previously unidentified clade, U1. *Synechococcus* communities at BATS DCM depths were more variable, showing greater contributions of clade II during the Oct. 2012 cruise (BATS286) and clades II and XVI during the July-2012 (BATS 283) cruise and the autumn BVAL cruise. Prior clade-specific niche determinations have shown that the SML-dominant clade III is considered to be an oligotrophic clade, while clade II is more broadly distributed in the tropics (Zwirgmaier et al., 2008), and clade XVI is more restricted to deep water (Ahlgren and Rocap, 2006).

Discussion

The results of this study show that measurable Si was present in nearly all *Synechococcus* cells analyzed, confirming that Si accumulation is a common

phenomenon in natural cells from the oligotrophic gyres. However, Si contents were highly variable and generally uncorrelated with measured environmental variables, suggesting that less direct effects such as community structure may drive Si accumulation in these ecosystems.

Si quotas and elevation in the SML

Previous reports of Si accumulation by *Synechococcus* in natural systems are limited to one station in the equatorial Pacific and one cruise in the Sargasso Sea (Baines et al., 2012). To expand these environmental observations in the oligotrophic gyre, we collected *Synechococcus* cells from the BATS site near Bermuda at several times in summer and fall (July through October) and from several sites between Bermuda and Puerto Rico in early October 2013 [Fig. 1]. Si quotas reported here, while variable in range, are consistent with previously reported field Si quotas in *Synechococcus* from the Sargasso Sea (means: 5 – 413 amol Si cell⁻¹) (Baines et al., 2012).

Single-cell SXRF analyses focused on cells collected from two depths: the surface mixed layer (SML; 5 – 10 m sampling depth) and the deep chlorophyll maximum (DCM: 75 – 88 m sampling depth) [Table 2] across a range environmental parameters that may have relationships to cellular Si. In one of the few consistent effects on field Si accumulation, despite its wide range and the narrow range in dissolved silicic acid concentrations, cells from SML had an average of two-fold more Si than cells from the DCM. Other environmental parameters, such as higher cell abundances, may have been linked to lower Si quotas due to growth dilution, while higher bSi concentrations or small-size bSi fractionation may have directly represented higher Si quotas in small *Synechococcus* cells. However, none of these environmental parameters acted as a significant predictor of cellular Si content [Fig. 3] within these oligotrophic ecosystems.

The difference in Si quotas between the SML and the DCM does not appear to be due to growth dilution. While we did not directly assess *Synechococcus* growth rates during the cruises, pigment-based estimates of cyanobacterial growth just north of BATS at the Ocean Flux Program site have demonstrated that cyanobacterial growth rates in the summer are highest in the SML and decline with depth (Goericke, 1998). Likewise, field measurements at station ALOHA have also shown that *Synechococcus* growth rates are typically higher (or similar) in the SML than at the DCM (Liu et al., 1995). When grown under different light levels in culture, *Synechococcus* clones from four different major sub-clades (Scanlan et al., 2009) exhibited an inverse correlation between Si content and growth rate (Brzezinski et al., 2016). Thus depth-related growth rates patterns of *Synechococcus* cells should have resulted in higher Si contents for DCM cells than in the SML, the opposite of what was observed. It appears then that growth-rate dilution is not likely the key determinant of depth-related differences in Si content.

Shifts in cyanobacterial community structure may be better related to the observed differences in *Synechococcus* Si content across depths, as *Synechococcus*

assemblages did markedly differ between the SML and the DCM [Fig. 4]. Experiments with cultured *Synechococcus* have demonstrated that clone identity can be a stronger predictor of Si quota than growth rate (Brzezinski et al., 2016). Assuming the recovered sequences reflect general shifts in *Synechococcus* assemblage structure, we were most likely to collect SXRF data from clade III *Synechococcus* in SML samples and from a greater proportion of clade II, XVI, and other *Synechococcus* clades in DCM samples. Community variation in Si uptake or sequestration provides a possible rationale for the observed differences in Si content between the SML and the DCM.

Variations in P and S quotas and biomass-normalization

Synechococcus P quotas reported here are similar to those previously reported at BATS (Twining et al. 2010; 20 – 51 amol P cell⁻¹) and in cultured strains (Bertilsson et al. 2003, Heldal et al. 2003; 15 – 116 amol P cell⁻¹). S quotas were also comparable to previous SXRF measurements at BATS (Twining et al. 2010; 35 – 79 amol S cell⁻¹) and to electron microprobe measurements of S in cultured *Synechococcus* cells (Heldal et al. 2003; 72 amol S cell⁻¹, mean of strains WH8103 and WH7803). Even with the caveats about P and S that are explored below, GLM-estimated least square geometric mean ratios of Si:S and Si:P for individual stations and depths in Table 2 are similar to those previously reported in *Synechococcus* cells collected from the eastern tropical North Pacific and the Sargasso Sea (Baines et al., 2012).

Ideally, both of the essential elements P and S can be used for biomass normalization, i.e. to compensate for any natural community variations in cell size. Biomass normalization for such a highly adaptable and cosmopolitan organism as *Synechococcus* must be considered carefully, however. Even essential elemental behaviors may be affected by independent physiological processes, some of which may be strain-dependent (as previously suggested for Si). Despite the environmental context provided by our field data, the physiological processes driving elemental accumulation in *Synechococcus* do remain rather unconstrained.

In a previous study of *Synechococcus* field assemblages that measured P and S quotas, Twining et al. (2010) found that P content was relatively invariant with depth, even across mesoscale eddy features that perturbed other elemental quotas. This result is replicated here for P, which was not significantly variant across SML and DCM depths [Table 3; Fig 2.]. We observed elevated S quotas in SML cells, also previously reported, which has been posited to reflect elevated protein content and/or glutathione reductant concentrations (both providing S-enrichment) in oxidatively-stressed cells from the upper euphotic zone (Dupont et al., 2004; Twining et al., 2010). In the P-stressed, if not P-limiting, North Atlantic surface waters of this study (Lomas et al., 2014; Mather et al., 2008), substitution of S for P in phospholipids may also contribute to higher S quotas in the SML (Van Mooy et al., 2009). Substitution of this type may be especially noticeable in picocyanobacteria with high surface area-to-volume (cell membrane-to-biomass) ratios in a region where comparatively scarce P is highly economized.

Quotas of S were higher in the SML than at the DCM, similar to trends in Si quotas. Despite station and cruise also being significant effects for S quotas [Table 3], Si:S ratios were insensitive to all effects (range: 0.4 – 3.8 mol:mol) [Table 2]. Our data cannot provide a clear physiological mechanism to link Si accumulation and S-indicated biomass, but do suggest that SML cells with higher Si and S contents may be larger and/or S-rich, while DCM cells with lower Si and S contents may be smaller and/or S-poor. The cause for any such relationship between Si and *Synechococcus* cell biomass, as indicated by invariant Si:S ratios, may ultimately be related to presently unquantified parameters such as clade-specific physiology and the underlying mechanisms of cellular Si acquisition and retention.

Quotas of P show no influence of a depth effect but do show a relationship with cruise (lower P quotas during the July/BATS283 cruise) which, in addition to any unquantified clade-specific physiological effects, may reflect changes in P availability and/or utilization at different times of year. Dissolved macronutrient concentrations measured by the BATS program are, however, typically below detection in the upper water column due to the ultra-oligotrophic conditions throughout the sampling periods. Unlike ratios of Si:S, ratios of Si:P show significant effects for cruise and depth, with SML cells possessing elevated Si:P [Table 2], further indicating additional accumulation of Si in the SML relative to P-indicated biomass. An important caveat is that approximately 25% of P measurements were below detection by SXRF, especially during the mid-summer BATS283 cruise (14 of 52 cells were below detection for P), potentially cautioning the universal usage of P as a biomass normalizer despite its relative invariance across SML and DCM ecosystems.

Contributions of Synechococcus Si to the bSi pool

We calculated potential contributions of *Synechococcus* Si to the 0.4 – 3 μm (small) bSi pool at each station and depth [Table 2, rightmost column] by multiplying the depth-specific (SML or DCM) distribution of cellular Si quotas [Fig 2] by the local *Synechococcus* cell abundances, then normalizing to locally measured small bSi. Estimates ranged over 30-fold, from 2 to 63% of the small bSi pool, though these contributions were always $\leq 8\%$ of total bSi. Greater potential contributions to small bSi that were observed during the autumn cruises reflected the lower bSi concentrations during the autumn bSi minimum. Lesser contributions were associated with the BATS283 cruise during which the station was occupied by a mesoscale eddy which resulted in enhanced diatom biomass [Table 2]. While most bSi is present in the larger size fraction, it appears there is demonstrable, albeit highly variable, potential for *Synechococcus* Si accumulation to contribute to the small bSi size-fraction. Consideration of additional processes such as aggregation and cycling of detrital material, diatom quotas, and grazing will be required to constrain the effects of *Synechococcus* on the total bSi cycle of these ecosystems.

Potential causes for Si variability in Synechococcus

What is driving the wide variability in Si quotas? Culture work has demonstrated that *Synechococcus* lacks an absolute Si requirement: strains show no growth reduction at zero-added Si ($\approx 1 \mu\text{M}$ ambient Si in Sargasso seawater culturing media) and no growth enhancement at higher Si concentrations (Brzezinski et al., 2016). Nevertheless, natural cells analyzed by SXRF have nearly all accumulated Si to the amol cell^{-1} level even in the low ambient silicic acid concentrations of the Sargasso Sea, with some cells accumulating many thousands of amol. For spherical cells with an approximate diameter of 1 micron, even 1 amol of Si accumulation is equivalent to $\approx 2 \text{ mM}$ (i.e. $\text{Si}(\text{OH})_4$ saturation in seawater) intracellular Si, indicating Si accumulation against a strong concentration gradient (ambient dissolved silicic acid: $0.6 - 1.3 \mu\text{M}$). This counter-concentration gradient argument—which would also apply to diatoms with their tens to hundreds of mM of intracellular Si, e.g. (Martin Jézéquel et al., 2000)—presumes that Si is kept in solution as silicic acid, which is not yet clear for *Synechococcus*. Accumulation may be enhanced by conversion to insoluble particulate forms, either biologically mediated or abiotically, the density effects of which are examined later.

Several possibilities for Si accumulation can be considered via comparison to other elemental behaviors. The biologically essential element P exhibits a much narrower range in cell quotas than Si [Fig. 2], with S quotas ranging somewhat intermediate to Si and P. For all three elements, population variability is attributed mostly to cells with higher quotas, as low-end quotas are on the order of $1 - 10 \text{ amol cell}^{-1}$ for all three elements. In other words, most quota distributions have a notable positive skew even after logarithmic transformation. The phenomenon of high-quota cells driving population heterogeneity has been shown previously in a diatom growth model for P utilization when modeled under conditions of non-limiting nutrient availability (Fredrick et al., 2013). Such results are consistent with Si's putatively non-essential nature in *Synechococcus* and also the general availability (non-limiting nature) of S in the marine environment.

Major drivers of heterogeneity implicated in the Fredrick et al. diatom growth model were: physiological maximum nutrient content per cell (i.e. storage capacity, or size); and how nutrients are partitioned to daughter cells during cell division (heterogeneity in growth dilution). Populations can also show heterogeneity in cell quotas due to variability in dissolved nutrient fields, an effect whose scale (factor of two variability in dissolved silicic acid) is dwarfed by the scale in Si quota variability (four orders of magnitude) [Table 2]. In the absence of strong heterogeneity in ambient silicic acid concentrations, unassessed nutrient concentrations in microenvironments on the scale of the cell (e.g. proximity to Si-rich diatoms or marine snow aggregates with hundreds of micromolar dissolved Si (Brzezinski et al., 1997)) may nevertheless affect the availability of elements to individual organisms. Other sources of quota heterogeneity may include different elemental requirements or accumulation tendencies by different ecotypes/clades [e.g. Fig. 4], and two possibilities not discernable in our dataset: variations in quotas throughout the cell cycle; or uptake in response to specific stressors. Given the apparent non-essential nature of Si, at least in the six cultured *Synechococcus* strains examined by

Brzezinski et al. (2016) and the element's as-yet-unclear uptake and storage mechanics, all possibilities remain potential explanations for the wide quota ranges for Si observed in natural *Synechococcus*.

Intracellular versus extracellular Si

A study by Tang et al. (2014) recently demonstrated Si accumulation within the extracellular polymeric substances (EPS) associated with decomposing *Synechococcus* cultures and in natural amorphous “micro-bleb” particles collected near Bermuda. Their results implicated abiotic or microbially mediated decomposition that drives Si localization to EPS during the aging and vertical export of *Synechococcus*-associated Si. Our observations on fresh cells, by comparison, demonstrate consistent Si accumulation by intact *Synechococcus* cells before decomposition. The spatial resolution of the SXRF cellular mapping methodology used here, though quantitative across the entire cell, is nevertheless insufficient to demonstrate where Si is physically located (i.e. intracellular versus extracellular). Improvements in quantitative, nanometer-scale SXRF mapping may soon allow quantitative extracellular/intracellular discernment on such small organisms (Deng et al., 2015a). The particulate speciation of Si in *Synechococcus* cultures examined via alternative x-ray methods is also the focus of a forthcoming manuscript by our group.

Effects of Si on cell density

To calculate maximum potential effects of Si accumulation on cell density, we can assume that all Si is present as amorphous opal (formula $\text{SiO}_2 \cdot 1.5\text{H}_2\text{O}$, 87.1 g mol^{-1} , density 2.1 g cm^{-3}). In an “average” cell, all 47 amol Si would equate to 4.1 fg opal with a volume of 2.0 aL. The combined, silicified excess cell density is less than 1% greater than the unsilicified cell, with opaline Si representing $\approx 0.8 \%$ of the average silicified cell's mass. For an upper-quartile cell with 1000 amol opaline Si, the effect on cell excess density increases: 14% of the cell mass as Si, with a silicified cell density $\approx 7\%$ greater than an unsilicified cell. While the excess density imparted by putatively opaline Si mass is non-negligible, neither “average” nor highly silicified cells would be expected to have sinking rates estimated using Stokes' law of $> 2 \text{ mm d}^{-1}$ [Table 4]. While it thus appears unlikely that silicification affects the sinking rates of individual *Synechococcus* cells, significantly greater sinking rates would be expected for larger aggregates or fecal pellets. Larger aggregates that have accumulated similar levels of excess density, either via silicified cells or through their own accumulation Si from seawater (Tang et al., 2014) would settle much more rapidly.

Conclusions

It remains surprising that *Synechococcus*, which has no known physiological use for Si and lacks a clear Si requirement in cultured strains, nevertheless accumulates this element to intracellular concentrations comparable to, and often exceeding, biologically essential P and S. Our data show that Si accumulation is a common feature of *Synechococcus* communities in oligotrophic regions. Cellular quotas of Si were similar, but highly variable, across several stations in the North Atlantic

occupied at different times of year and in different years. Si quotas showed no consistent relationship with ambient bSi concentrations or *Synechococcus* abundances but may contribute significantly to the small ($< 3 \mu\text{m}$) bSi pool. Even within the low and narrow silicic acid concentrations of the euphotic oligotrophic gyre, Si content in cells from the SML was nearly double that of cells from the DCM. In the low Si concentrations of the oligotrophic gyres, parameters other than bulk Si availability—such as shifts in *Synechococcus* community structure—may exert control over highly variable cellular Si contents. While the potential presence of solid silica phases in individual cells is unlikely to provide enough excess density to impart significant vertical settling speeds on single living cells, Si accumulation may influence settling speeds in larger aggregates composed of such cells.

Acknowledgments

We thank the BATS program for cruise access and E. Lachenmyer, I. Marquez, C. Griffin for shipboard assistance. This project was funded by the National Science Foundation to BST (OCE 1131046), JWK and MAB (OCE 1335012) and SBB and JLC (OCE 1131139). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

Figure and Table Captions

Table 1: Synchrotron analytical run metadata: run number, analyzed cruise, and typical cell region of interest (ROI) elemental uncertainties for Si, P and S (median $\pm$ 1 s.d., in percent). Elemental background levels vary across samples, runs, and even within sample grids, so in lieu of detection limits, typical (median) cell-to-background ROI ratios are provided. Additional methodological information is provided in the text.

Table 2: SXRF-measured single-cell Si quotas and biomass ratios along with key environmental parameters measured from the same bottles. SML: surface mixed layer (5 – 10 m sampling depth). DCM: deep chlorophyll maximum (75 – 88 m sampling depth). BATS site located at $31^{\circ}40'N/64^{\circ}10'W$. Asterisks (*): Least-square means and std. errors from the GLM described in the text. Bold: cast instead of station number. *Italics* (BATS-286, Cast 1, DCM): informational SXRF values only due to the few number of samples analyzed.

Table 3: Within a generalized linear model containing the specified effects (cruise, station nested within cruise, and depth), the degrees of freedom (DF) and significance of the effects (p-values) on elemental quotas ($\log_{10}$ element/cell) and quota ratios. Bold values: effects significant at the $p < 0.05$ level.

Table 4: Maximum sinking speeds estimated via Stokes' Law for spherical particles of various diameters that have accumulated amorphous opal Si, and thus excess density, at mean ($47 \text{ amol Si cell}^{-1}$) and upper-quartile ($1000 \text{ amol Si cell}^{-1}$) levels. Settling speeds of individual silicified *Synechococcus* cells ($1 \mu\text{m}$ diameter) are ≤ 2

mm d⁻¹, but larger aggregates of similar excess density could settle much more rapidly.

Figure 1: Locations of major sampling stations in the western Atlantic.

Figure 2: Histograms of *Synechococcus* Si, P, and S quotas in amol cell⁻¹ (note matched log y-axis scales). Data are from all cruises and stations, binned by depth: left, surface mixed layer (SML; n=73); right, deep chlorophyll maximum (DCM; n=41). Asterisks: LSQ-geometric-means indicated by dashed lines are significantly different between SML and DCM for Si and S, but not for P.

Figure 3: Relationships between LSQ-geometric-mean SXRF cellular Si quotas (y-axes) and various environmental parameters (x-axes) were not significant (best fit lines, Pearson *r* and p-values; n=11). Blue points: SML. Green points: DCM.

Figure 4: *Synechococcus* operational taxonomic unit (OTU) contributions by various identified/cultured clades (Mazard et al., 2012) and two unnamed clusters (U1 and U2) to communities from the surface mixed layer (SML; 5 – 10 m sampling depth) and deep chlorophyll maximum (DCM; 75 – 88 m sampling depth).

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J.: Basic local alignment search tool, *Journal of Molecular Biology*, 215(3), 403–410, doi:10.1016/S0022-2836(05)80360-2, 1990.
- Baines, S. B., Twining, B. S., Brzezinski, M. A., Krause, J. W., Vogt, S., Assael, D. and McDaniel, H.: Significant silicon accumulation by marine picocyanobacteria, *Nature Geoscience*, 5(12), 886–891, doi:10.1038/ngeo1641, 2012.
- Bertilsson, S., Berglund, O. and Karl, D. M.: Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea, *Limnology and Oceanography*, 2003.
- Brzezinski, M. A. and Nelson, D. M.: The annual silica cycle in the Sargasso Sea near Bermuda, *Deep Sea Research Part I: Oceanographic Research Papers*, 42(7), 1215–1237, doi:10.1016/0967-0637(95)93592-3, 1995.
- Brzezinski, M. A., Alldredge, A. L. and O'Bryan, L. M.: Silica cycling within marine snow, *Limnology and Oceanography*, 42(8), 1706–1713, doi:10.4319/lo.1997.42.8.1706, 1997.
- Brzezinski, M. A., Krause, J. W., Baines, S. B., Collier, J. L., Ohnemus, D. C. and Twining, B. S.: Patterns and Regulation of Silicon Accumulation in *Synechococcus* spp., *Limnology and Oceanography* (in review). 2016.

636 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E.
637 K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T.,
638 Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D.,
639 Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J.,
640 Yatsunencko, T., Zaneveld, J. and Knight, R.: QIIME allows analysis of high-throughput
641 community sequencing data, *Nat Meth*, 7(5), 335–336, doi:10.1038/nmeth.f.303,
642 2010.

643 Collier, J. L. and Palenik, B.: Phycoerythrin-containing picoplankton in the Southern
644 California Bight, *Deep Sea Research Part II: Topical Studies in Oceanography*, 2003.

645 Deng, J., Vine, D. J., Chen, S., Nashed, Y. S. G., Jin, Q., Phillips, N. W., Peterka, T., Ross, R.,
646 Vogt, S. and Jacobsen, C. J.: Simultaneous cryo X-ray ptychographic and fluorescence
647 microscopy of green algae, *Proceedings of the National Academy of Sciences*, 112(8),
648 2314–2319, doi:10.1073/pnas.1413003112, 2015a.

649 Deng, W., Monks, L. and Neuer, S.: Effects of clay minerals on the aggregation and
650 subsequent settling of marine *Synechococcus*, *Limnology and Oceanography*, 60(3),
651 805–816, doi:10.1002/lno.10059, 2015b.

652 Dupont, C. L., Goepfert, T. J., Lo, P. and Wei, L.: Diurnal cycling of glutathione in
653 marine phytoplankton: Field and culture studies, *Limnology and Oceanography*,
654 2004.

655 Edgar, R. C.: Search and clustering orders of magnitude faster than BLAST,
656 *Bioinformatics*, 26(19), 2460–2461, doi:10.1093/bioinformatics/btq461, 2010.

657 Fredrick, N. D., Berges, J. A., Twining, B. S., Nuñez-Milland, D. and Hellweger, F. L.:
658 Use of Agent-Based Modeling To Explore the Mechanisms of Intracellular
659 Phosphorus Heterogeneity in Cultured Phytoplankton, *Applied and Environmental*
660 *Microbiology*, 79(14), 4359–4368, doi:10.1128/AEM.00487-13, 2013.

661 Goericke, R.: Response of phytoplankton community structure and taxon-specific
662 growth rates to seasonally varying physical forcing in the Sargasso Sea off Bermuda,
663 *Limnology and Oceanography*, 43(5), 921–935 [online] Available from:
664 <http://onlinelibrary.wiley.com/doi/10.4319/lo.1998.43.5.0921/full>, 1998.

665 Guidi, L., Chaffron, S., Bittner, L., Eveillard, D. and Larhlimi, A.: Plankton networks
666 driving carbon export in the oligotrophic ocean, *Nature*, doi:10.1038/nature16942,
667 2016.

668 Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M., Ward, D. V., Giannoukos, G., Ciulla,
669 D., Tabbaa, D., Highlander, S. K., Sodergren, E., Methe, B., DeSantis, T. Z., The Human
670 Microbiome Consortium, Petrosino, J. F., Knight, R. and Birren, B. W.: Chimeric 16S
671 rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR
672 amplicons, *Genome Research*, 21(3), 494–504, doi:10.1101/gr.112730.110, 2011.

673 Hall, T. A.: BioEdit: a user-friendly biological sequence alignment editor and analysis
674 program for Windows 95/98/NT, Nucleic acids symposium series, 1999.

675 Heldal, M., Scanlan, D. J., Norland, S., Thingstad, F. and Mann, N. H.: Elemental
676 composition of single cells of various strains of marine *Prochlorococcus* and
677 *Synechococcus* using X-ray microanalysis, *Limnology and Oceanography*, 48(5),
678 1732–1743, 2003.

679 Krause, J. W., Brzezinski, M. A. and Landry, M. R.: The effects of biogenic silica
680 detritus, zooplankton grazing, and diatom size structure on silicon cycling in the
681 euphotic zone of the eastern equatorial Pacific, *Limnology and Oceanography*, 2010.

682 Krause, J. W., Lomas, M. W. and Nelson, D. M.: Biogenic silica at the Bermuda Atlantic
683 Time-series Study site in the Sargasso Sea: Temporal changes and their inferred
684 controls based on a 15-year record, *Global Biogeochem. Cycles*, 23(3),
685 doi:10.1029/2008GB003236, 2009.

686 Liu, H., Campbell, L. and Landry, M. R.: Growth and mortality rates of
687 *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique,
688 *Marine Ecology Progress Series*, 1995.

689 Lomas, M. W., Bonachela, J. A., Levin, S. A. and Martiny, A. C.: Impact of ocean
690 phytoplankton diversity on phosphate uptake, *Proceedings of the National Academy*
691 *of Sciences*, 111(49), 17540–17545, doi:10.1073/pnas.1420760111, 2014.

692 Lomas, M. W., Steinberg, D. K., Dickey, T., Carlson, C. A., Nelson, N. B., Condon, R. H.
693 and Bates, N. R.: Increased ocean carbon export in the Sargasso Sea linked to climate
694 variability is countered by its enhanced mesopelagic attenuation, *Biogeosciences*,
695 7(1), 57–70, doi:10.5194/bg-7-57-2010, 2010.

696 Marquez, I. A.: The consequences of mesoscale eddies on diatom production and the
697 coupling of Si and organic matter export in the Sargasso Sea, University of South
698 Alabama. 2015.

699 Martin Jézéquel, V., Hildebrand, M. and Brzezinski, M. A.: Silicon metabolism in
700 diatoms: implications for growth, *Journal of Phycology*, 36(5), 821–840, 2000.

701 Mather, R. L., Reynolds, S. E., Wolff, G. A. and Williams, R. G.: Phosphorus cycling in
702 the North and South Atlantic Ocean subtropical gyres, *Nature Geoscience*,
703 doi:10.1038/ngeo232, 2008.

704 Mazard, S., Ostrowski, M., Partensky, F. and Scanlan, D. J.: Multi-locus sequence
705 analysis, taxonomic resolution and biogeography of marine *Synechococcus*,
706 *Environmental Microbiology*, 14(2), 372–386, doi:10.1111/j.1462-
707 2920.2011.02514.x, 2012.

708 Núñez-Milland, D. R., Baines, S. B., Vogt, S. and Twining, B. S.: Quantification of

709 phosphorus in single cells using synchrotron X-ray fluorescence, *Journal of*
710 *Synchrotron Radiation*, 17(4), 560–566, doi:10.1107/S0909049510014020, 2010.

711 Polovina, J. J., Howell, E. A. and Abecassis, M.: Ocean's least productive waters
712 are expanding, *Geophysical Research Letters*, 35(3), doi:10.1029/2007GL031745,
713 2008.

714 Saito, M. A., Rocap, G. and Moffett, J. W.: Production of cobalt binding ligands in a
715 *Synechococcus* feature at the Costa Rica upwelling dome, *Limnology and*
716 *Oceanography*, 50(1), 279–290, doi:10.4319/lo.2005.50.1.0279, 2005.

717 Scanlan, D. J., Ostrowski, M., Mazard, S., Dufresne, A., Garczarek, L., Hess, W. R., Post,
718 A. F., Hagemann, M., Paulsen, I. and Partensky, F.: Ecological Genomics of Marine
719 Picocyanobacteria, *Microbiology and Molecular Biology Reviews*, 73(2), 249–299,
720 doi:10.1128/MMBR.00035-08, 2009.

721 Stukel, M. R., Décima, M., Selph, K. E., Taniguchi, D. A. A. and Landry, M. R.: The role
722 of *Synechococcus* in vertical flux in the Costa Rica upwelling dome, *Progress in*
723 *Oceanography*, 112-113, 49–59, doi:10.1016/j.pocean.2013.04.003, 2013.

724 Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S.: MEGA6: Molecular
725 Evolutionary Genetics Analysis Version 6.0, *Mol Biol Evol*, 30(12), 2725–2729,
726 doi:10.1093/molbev/mst197, 2013.

727 Tang, T., Kisslinger, K. and Lee, C.: Silicate deposition during decomposition of
728 cyanobacteria may promote export of picophytoplankton to the deep ocean, *Nature*
729 *Communications*, 5, doi:10.1038/ncomms5143, 2014.

730 Twining, B. S., Baines, S. B., Bozard, J. B., Vogt, S., Walker, E. A. and Nelson, D. M.:
731 Metal quotas of plankton in the equatorial Pacific Ocean, *Deep Sea Research Part II:*
732 *Topical Studies in Oceanography*, 58(3-4), 325–341,
733 doi:10.1016/j.dsr2.2010.08.018, 2011.

734 Twining, B. S., Baines, S. B., Fisher, N. S., Maser, J., Vogt, S., Jacobsen, C., Tovar-
735 Sánchez, A. and Sañudo-Wilhelmy, S. A.: Quantifying Trace Elements in Individual
736 Aquatic Protist Cells with a Synchrotron X-ray Fluorescence Microprobe, *Analytical*
737 *Chemistry*, 75(15), 3806–3816, doi:10.1021/ac034227z, 2003.

738 Twining, B. S., Nunez-Milland, D. and Vogt, S.: Variations in *Synechococcus* cell
739 quotas of phosphorus, sulfur, manganese, iron, nickel, and zinc within mesoscale
740 eddies in the Sargasso Sea, *Limnology and Oceanography*, 2010.

741 Van Mooy, B., Fredricks, H. F., Pedler, B. E. and Dyhrman, S. T.: Phytoplankton in the
742 ocean use non-phosphorus lipids in response to phosphorus scarcity, *Nature*, 2009.

743 Vogt, S.: MAPS : A set of software tools for analysis and visualization of 3D X-ray
744 fluorescence data sets, *J. Phys. IV France*, 104, 635–638, doi:10.1051/jp4:20030160,

745 2003.

746 Wang, W. and Dei, R.: Bioavailability of iron complexed with organic colloids to the
747 cyanobacteria *Synechococcus* and *Trichodesmium*, *Aquatic Microbial Ecology*,
748 33(3), 247–259, 2003.

749

Analytical Run	Cruise	Median Cell ROI Uncertainty [percent $\pm$ 1 s.d.]			Median Cell/Bkgr ROI Ratio [percent]		
		Si	P	S	Si	P	S
2012-3	BATS283	4.7 $\pm$ 3.4	14 $\pm$ 14	2.6 $\pm$ 1.6	152	874	412
2013-1	BATS286	3.2 $\pm$ 3.3	10 $\pm$ 18	3.2 $\pm$ 2.6	127	404	424
2013-3	BVAL	8.1 $\pm$ 6.8	17 $\pm$ 11	5.4 $\pm$ 2.6	165	614	345

Table 1: Synchrotron analytical run metadata: run number, analyzed cruise, and typical cell region of interest (ROI) elemental uncertainties for Si, P and S (median $\pm$ 1 s.d., in percent). Elemental background levels vary across samples, runs, and even within sample grids, so in lieu of detection limits, typical (median) cell-to-background ROI ratios are provided. Additional methodological information is provided in the text.

Cruise	Cast/ Stn	Depth	Location	Date	Ambient SiOH ₄ (μM)	N Cells SXRf	Si (amol/cell) SXRf*	Si:P (mol:mol) SXRf*	Si:S (mol:mol) SXRf*	0.4 - 3 μm small bSi (nmol L ⁻¹)	>3 μm large bSi (nmol L ⁻¹)	%bSi small	Syn. cells/mL	Small bSi potentially Syn.
BATS-283	1	SML	BATS	11-Jul-2012	0.8	12	57 ± 2	3.8 ± 0.2	1.3 ± 0.2	39	139	22%	12,146	3%
		DCM			1.3	16	16 ± 1	0.6 ± 0.2	0.3 ± 0.2	11	140	7%	2,210	2%
	3	SML	BATS	12-Jul-2012	0.9	15	64 ± 1	3.7 ± 0.2	0.3 ± 0.2	14	203	6%	14,759	12%
		DCM			0.9	9	14 ± 1	1.1 ± 0.2	0.8 ± 0.2	8.8	143	6%	13,352	12%
BATS-286	1	SML	BATS	18-Oct-2012	0.8	10	42 ± 1	2.5 ± 0.2	0.9 ± 0.2	4.9	12	29%	12,638	29%
		DCM			0.6	2	37 ± 2	2.7 ± 0.3	3.2 ± 0.4	1.3	11	10%	1,775	11%
	3	DCM	BATS	19-Oct-2012	0.6	5	60 ± 2	1.6 ± 0.2	2.4 ± 0.3	2.3	12	16%	8,709	31%
BVAL	2	SML	BATS	30-Sep-2013	1.2	7	40 ± 2	1.7 ± 0.2	0.6 ± 0.2	2.1	13	14%	3,949	21%
		DCM			1.0	10	32 ± 2	0.4 ± 0.2	1.8 ± 0.3	1.7	13	12%	1,618	8%
	5	SML	28°39.8'N/64°36.7'W	1-Oct-2013	0.9	9	38 ± 2	0.5 ± 0.2	0.6 ± 0.2	1.3	9.8	12%	4,008	34%
	10	SML	23°10.1'N/65°21.9'W	3-Oct-2013	1.3	11	55 ± 2	2.3 ± 0.2	0.8 ± 0.2	1.7	15	10%	5,500	37%
	12	SML	21°40.2'N/65°40.0'W	4-Oct-2013	1.3	11	59 ± 2	2.8 ± 0.3	0.7 ± 0.2	1.4	13	10%	7,952	63%

Table 2: SXRf-measured single-cell Si quotas and biomass ratios along with key environmental parameters measured from the same bottles. SML: surface mixed layer (5 – 10 m sampling depth). DCM: deep chlorophyll maximum (75 – 88 m sampling depth). BATS site located at 31°40'N/64°10'W. Asterisks(*): Least-square means and std. errors from the GLM described in the text. Bold: cast instead of station number. *Italics* (BATS-286, Cast 1, DCM): informational SXRf values only due to the few number of samples analyzed.

Effect	DF	log Si/cell	log P/cell	log S/cell	log Si:P	log Si:S	log P:S
Cruise	2	0.723	0.008	0.002	0.029	0.051	<.0001
Station [Cruise]	5	0.840	0.067	0.037	0.089	0.301	0.004
Depth	1	0.029	0.288	<.0001	.0001	0.422	<.0001

Table 3: Within a generalized linear model (GLM) containing the specified effects (cruise, station nested within cruise, and depth), the degrees of freedom (DF) and significance of the effects (p-values) on elemental quotas ($\log_{10}$ element/cell) and quota ratios. Bold values: effects significant at the $p < 0.05$ level.

	amol Si·cell ⁻¹	
	47	1000
Particle diameter	Excess density as opal (kg·m ⁻³)	
	4	80
1 µm (<i>Syn. cell</i>)	0.0001	0.002
10 µm aggregate	0.01	0.2
100 µm aggregate	1	20
sinking speed (m·d⁻¹)		

Table 4: Maximum sinking speeds estimated via Stokes' Law for spherical particles of various diameters that have accumulated amorphous opal Si, and thus excess density, at mean (47 amol Si cell⁻¹) and upper-quartile (1000 amol Si cell⁻¹) levels. Settling speeds of individual silicified *Synechococcus* cells ($\approx 1\ \mu\text{m}$ diameter) are $\leq 2\ \text{mm d}^{-1}$, but larger aggregates of similar excess density could settle much more rapidly.

Figure 1: Locations of major sampling stations described in the text.

Si Quotas

Figure 2: Histograms of *Synechococcus* Si, P and S quotas in amol cell^{-1} (note matched log y-axis scales). Data are from all cruises and stations, binned by depth: left, surface mixed layer (SML; $n=73$); right, deep chlorophyll maximum (DCM; $n=41$). GLM-estimated least square geometric means are indicated as horizontal lines. Means for Si and S are statistically significantly different at the $p < 0.05$ level.

Figure 3: Relationships between LSQ-mean SXRF cellular Si quotas (y-axes) and various environmental parameters (x-axes) were not significant (best fit lines, Pearson r and p -values; $n=11$). Blue points: SML. Green points: DCM.

Figure 4: *Synechococcus* operational taxonomic unit (OTU) contributions by various identified, cultured clades (Mazard et al., 2012), and two unnamed clusters (U1 and U2), to surface mixed layer (SML; 5-10m) and deep chlorophyll maximum (DCM; 75-88m) communities.