Metal contents of phytoplankton and labile particulate material in the North Atlantic Ocean

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ABSTRACT

Phytoplankton contribute significantly to global C cycling and serve as the base of ocean food webs. Phytoplankton require trace metals for growth and also mediate the vertical distributions of many metals in the ocean. We collected bulk particulate material and individual phytoplankton cells from the upper water column (<150 m) of the North Atlantic Ocean as part of the US GEOTRACES North Atlantic Zonal Transect cruise (GEOTRACES GA03). Particulate material was first leached to extract biogenic and potentially-bioavailable elements, and the remaining refractory material was digested in strong acids. The cruise track spanned several ocean biomes and geochemical regions. Particulate concentrations of metals associated primarily with lithogenic phases (Fe, Al, Ti) were elevated in surface waters nearest North America, Africa and Europe, and elements associated primarily with biogenic material (P, Cd, Zn, Ni) were also found at higher concentrations near the coasts. However metal/P ratios of labile particulate material were also elevated in the middle of the transect for Fe, Ni, Co, Cu, and V. P-normalized cellular metal quotas measured with synchrotron X-ray fluorescence (SXRF) were generally comparable to ratios in bulk labile particles but did not show mid-basin increases. Manganese and Fe ratios and cell quotas were higher in the western part of the section, nearest North America, and both elements were more enriched in bulk particles, relative to P, than in cells, suggesting the presence of labile oxyhydroxide particulate phases. Cellular Fe quotas thus did not increase in step with aeolian dust inputs, which are highest near Africa; these data suggest that the dust inputs have low bioavailability. Copper and Ni cell quotas were notably higher nearest the continental margins. Overall mean cellular metal quotas were similar to those measured in the Pacific and Southern Oceans except for Fe, which was approximately 3-fold higher in North Atlantic cells. Cellular Fe quotas are in-line with those measured in laboratory cultures at comparable Fe concentrations. Particulate Zn, Cu, Ni, and Co are primarily associated with cellular material, but less than 30% of labile particulate Fe and Mn are biogenic. Particulate Al was primarily associated with lithogenic material, but the labile fraction was highly correlated with P, as well as with biogenic silica, suggesting that some particulate Al (perhaps around 20%) may occur adsorbed to biogenic material. Cellular element maps indicate that externally scavenged Fe was not a significant fraction of the metal associated with live phytoplankton, but adsorbed or precipitated phases are likely to be important in particulate detrital material. Such abiotic scavenging, along with differential remineralization of cellular nutrients in the water column, results in estimates of cellular metal/nutrient ratios from dissolved concentrations that significantly underestimate the ratios in phytoplankton. These data demonstrate the response of phytoplankton to the unique metal inputs to the North Atlantic Ocean.

KEYWORDS: GEOTRACES, biogeochemistry, scavenging, metal cycling, suspended particulate material

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1. INTRODUCTION

Phytoplankton contribute significantly to global C cycling and serve as the base of ocean food webs. A suite of transition metals are required as nutrients for phytoplankton (Sunda, 1988/1989). Iron is the most quantitatively important metal nutrient for marine plankton (Ho et al., 2003), but Mn, Co, Ni, Cu and Zn are typically also required for proper cell function (Frausto da Silva & Williams, 2001). Metal availability can control the growth of phytoplankton (Boyd et al., 2007; Moore et al., 2013), and controls on phytoplankton growth and metabolism can subsequently affect the biogeochemical cycles of other nutrient elements such as C, N, P, S, and Si (Morel & Price, 2003).

Trace metal concentrations in phytoplankton (also referred to as quotas) are poorly constrained in natural systems. Much of what is known about plankton metal quotas and stoichiometries comes from laboratory culture studies (e.g., Ho et al., 2003; Kustka et al., 2003; Sunda & Huntsman, 2000; Sunda et al., 1991). However our incomplete understanding of the relationship between metal speciation and bioavailability (Campbell, 1995; Campbell et al., 2002; Hudson, 1998), as well as frequently uncharacterized metal speciation in natural systems, limits our ability to apply laboratory results to natural systems. Additionally, metal quotas can vary significantly as a function of many environmental factors, including temperature (Miao & Wang, 2006), light intensity (Finkel et al., 2006; Sunda & Huntsman, 1997), photoperiod (Sunda & Huntsman, 2004) and macronutrient availability (Maldonado & Price, 1996). Trace metal quotas have also been shown to vary between different taxonomic phytoplankton groups grown under the same conditions (Quigg et al., 2003). Given the complex and dynamic environmental conditions experienced by phytoplankton growing in the ocean, direct measurements of metal quotas or stoichiometries in natural plankton communities are needed to advance our understanding of metal biogeochemistry.

Bulk measurements of particulate metals in size-fractionated samples are of limited value for exploring phytoplankton metal quotas due to relatively high concentrations of metals in co-occurring abiotic particles. Previous bulk studies of phytoplankton metal contents have typically been limited to remote low-metal regions of the ocean (Collier & Edmond, 1984; Kuss & Kremling, 1999). More recent studies have employed single-cell elemental measurements via synchrotron x-ray fluorescence (SXRF) microscopy, and this has enabled advances in our understanding of metal quotas in natural phytoplankton populations. Previous SXRF studies have focused on mostly low-Fe environments such as the Southern Ocean and equatorial Pacific Ocean (Twining et al., 2011; Twining et al., 2004a), while SXRF studies in the Atlantic Ocean have focused on individual taxa only in the Sargasso Sea (Nuester et al., 2012; Twining et al., 2010).

In this study bulk particles and individual phytoplankton cells were collected as part of the US GEOTRACES North Atlantic Zonal Transect (NAZT) cruise (GA03). The cruise track spanned several oceanographic biomes and geochemical regions, providing opportunities for examining changes in cellular trace element (TE) quotas and biogenic particulate TE. Much of the cruise was in the subtropical latitudes that are characterized by high aeolian inputs from North Africa. This study presents a first opportunity to examine the metal quotas of phytoplankton within the North Atlantic Ocean, focusing on comparisons between bulk and single-cell metal ratios, the biogeochemical implications of observed differences, as well as comparisons with cells from

other ocean basins. We also examine evidence for metal scavenging in the upper water column. All data reported here are available through the Biological and Chemical Oceanography Data Management Office (<http://www.bco-dmo.org/>).

2. MATERIALS AND METHODS

2.1 Sample collection

Samples were collected during two US GEOTRACES NAZT cruises in 2010 and 2011 (Fig. 1A). The 2010 cruise sampled from the Mediterranean outflow near Lisbon to the upwelling system off West Africa to the Cape Verde Islands during late October. The 2011 cruise sampled from the North American shelf to the Cape Verde Islands during late November and early December. Particulate samples were collected from GO-FLO bottles at every station and through the full depth of the water column, however only data for the upper 150 m are presented here (remaining samples are archived). Single-cell SXRF samples were collected from the surface mixed layer (ML) and deep chlorophyll maximum (DCM) at most stations, however access to synchrotron beamtime limited analyses to samples from a subset of 10 stations (Fig. 1A, Table 1) that were chosen either as representative of an ocean region (i.e., continental slope, upwelling region, sub-tropical gyre) or due to good cell abundance in SXRF samples. Additional surface samples were collected with the ‘GeoFish’ (Bruland et al., 2005) during a transect from the Cape Verde Islands to Charleston, SC, in November following engine problems on the ship that ended the 2010 cruise, and we have included SXRF data for two of these stations (99 and 153; Table 1) in order to augment the relatively small number of SXRF samples.

2.2 Preparation and analysis of bulk particle samples

Collection and analysis of particulate samples is described in Twining et al. (2014b). Briefly, particles were collected onto 25-mm diameter Supor 0.45- μ m porosity polyethersulfone filters via pressurization of GO-FLO bottles with 0.2- μ m filtered air. Filtration was continued until the entire bottle was empty or 2 hours had elapsed to limit loss of larger particles that may settle in the bottle during filtration (Planquette & Sherrell, 2012). The filtrate was collected for measurement of filtration volume. An average of 6.5-L of water was filtered through each filter. Filter holders were removed from the GO-FLO bottles and a vacuum applied to remove residual water. Filters were then folded, stored in acid-washed centrifuge tubes, and frozen at -20°C until digestion and analysis on shore. Digestion and analysis of particles was performed under Class-100 conditions. Filters from the ML and DCM bottles were digested in 25% acetic acid and 0.02 M hydroxylamine hydrochloride in the polypropylene centrifuge tubes used for storage, following the protocol of Berger et al. (2008). Filters were then transferred to acid-cleaned 22-mL PFA digestion vials (Savillex) and subsequently digested using the mixture of concentrated acids described below to recover the refractory elements (see details below). The acetic acid/hydroxylamine leachate was centrifuged to sediment any remaining particles and the leachate supernatant transferred into a clean digestion vial. Nitric acid was added and the vial was heated uncapped to near dryness. Vial contents were redissolved with 2% HNO₃. A complete evaluation of the Berger et al. (2008) leach, including intercomparison with other approaches to estimating the biogenic and labile fractions, is presented elsewhere (Rauschenberg & Twining, 2015).

The refractory particulate fraction was digested in a mixture of 4M HCl, 4M HNO₃, and 4M HF. Following the labile digest, the filter and any remaining leachate were transferred to a clean PFA

vial, 2-mL of acid mixture was added, and the vial was tightly capped and heated to 110°C for 4 hours. This procedure has been determined to be adequate for digestion of all particulate material, while allowing the Supor filter to remain largely intact (Ohnemus et al., 2014). Following heating, the acid solution in the bomb was transferred to a clean PFA vial along with 3 rinses of ultrapure water. This vial was then heated to dryness and the contents re-dissolved in a mixture of 50% HNO₃ + 15% H₂O₂. This solution was again dried down and the contents re-dissolved with 2% HNO₃. A complete description of the procedure is presented elsewhere (Twining et al., 2014b).

All bottle sample digests were analyzed using a Thermo Scientific Element2 magnetic sector ICP-MS at the University of Maine following the protocols outlined in Twining et al. (2011). The instrument was equipped with an ESI Apex desolvation nebulizer, an autosampler contained in a clean bench, and Ni cones. Quantification was performed by 3-point standard additions, and In-115 was used as an internal standard to correct for variations in instrumental sensitivity during analyses. Cd-111 was analyzed in low-resolution mode, and the remaining isotopes (Al-27, P-31, Ti-47, V-51, Mn-55, Fe-56, Fe-57, Co-59, Ni-60 Cu-63, Zn-66) were analyzed in medium-resolution mode. When multiple isotopes were analyzed for some elements (e.g., Fe-56, Fe-57), concentrations were within 4%, on average (concentrations of Fe-56 are reported here).

2.3 Preparation and analysis of SXRF samples

Unfiltered water samples from the ML and DCM were collected from GO-FLO bottles prior to particle filtration. Cells were preserved with 0.25% trace-metal clean buffered glutaraldehyde (Twining et al., 2003) and centrifuged onto C/formvar-coated Au or Al TEM grids. As performed previously (e.g., Twining et al., 2011), Au grids were briefly rinsed with a drop of ultrapure water and dried in a Class-100 cabinet. Aluminum grids were also used at some stations in an effort to obtain accurate data on Cu contents of plankton cells, since Au grids contain a Cu-alloy which results in significant Cu contamination of cells (Twining et al., 2003). Aluminum grids were rinsed with an ammonium formate solution (0.8 M, pH 8; precleaned using Chelex-100) to further decrease the leaching of Cu from the grids. A subset of both Au and Al grids were treated with a Chelex-cleaned poly-l-lysine solution (0.01%) prior to cell mounting to improve cell adhesion to grids (Marchant & Thomas, 1983). Preliminary tests of poly-l-lysine coated grids indicated no contamination from this step.

Mean element quotas normalized to cell biovolume were compared for cells mounted on Au or Al grids. Cells mounted on Al grids had significantly ($p < 0.01$) lower concentrations of Cu, as expected, along with significantly higher concentrations of S, Mn, Fe and Ni. Direct analysis of the Al grids showed them to contain significant Mn and Fe; therefore the higher cell quotas of these metals likely result from grid contamination. The cause of the difference in S quotas is unknown, but given the good agreement of S quotas for Au-mounted cells with expectations, as well as the extensive use of these grids in the past (Twining et al., 2011; Twining et al., 2004a; Twining et al., 2003), it is concluded that the S quotas for Al-mounted cells are not reliable. The discrepancy in Ni quotas may result from overcorrection of Cu fluorescence on the Au grids rather than Ni contamination from the Al grids, since SXRF analyses show negligible Ni content in the Al grids. The Ni K_α peak occurs on a shoulder of the Cu K_α peak and therefore can be influenced by the Cu K_α peak. Therefore, data for Mn, Fe, Co, and Zn are taken from Au-mounted cells, while Ni and Cu data are from Al-mounted cells.

SXRF analysis was performed using the 2-ID-E beamline at the Advanced Photon source (Argonne National Laboratory) following the protocols of Twining et al. (2011). Samples were analyzed during runs in July 2011 and August 2012. Between 8 and 34 cells were analyzed from each station-depth combination (Table 1). Each cell was raster scanned with a focused 10 keV x-ray beam with a diameter of approximately 0.5 μm . Step sizes were 0.4 μm , and dwell times at each pixel were 1-4 sec, depending on the signal intensity. Fluorescence counts were normalized to dwell time and incident beam intensity. Fluorescence spectra from the pixels covering the cell were averaged to calculate whole-cell quotas, and a fluorescence spectrum from a neighboring empty section of the grid was subtracted. Cellular K_{α} fluorescence intensities for Si, P, S, Mn, Fe, Co, Ni, Cu, and Zn were then fit with a modified-Gaussian model using custom software and peak areas converted to areal element concentrations using NBS-certified standard reference materials (Núñez-Milland et al., 2010; Twining et al., 2011). Spatial regions of interest (ROI) representing the whole cell (including any adsorbed elements or flagella, if present) were prepared for each cell and used to calculate quotas.

SXRF measurements of individual cells have been shown previously to have a relative precision of approximately 5% (Twining et al., 2003), and propagated uncertainty for P- and S-normalized metal ratios are expected to be less than 10% (Twining et al., 2011). Four cells—autotrophic flagellates between 3 and 14 μm —were analyzed multiple times (2-4 times) in order to check precision of the SXRF analyses during these runs. Replicate analyses varied on average by approximately 3% for P and S. Precision for trace metal measurements depended on signal strength in each cell, but were approximately 10% for Mn, Fe, Ni, Cu and Zn. Precision of metal measurements was better than 2% for larger cells (i.e., >10 μm in diameter). These results are comparable to those reported previously for this technique (Twining et al., 2003). The precision of Co measurements is affected by the relative abundance of Fe, due to overlapping Fe and Co fluorescence peaks. Precision of Co quotas is limited to approximately 50%.

Cell biovolume was calculated for each cell from measurements of cell length and width using digital image processing software (ImagePro Plus). Shape and volume equations were taken from Hillebrand et al. (1999). Cellular C was calculated from cell volume using the equations of Menden-Deuer and Lessard (2000) as during previous work (Twining et al., 2011; Twining et al., 2004a). SXRF analysis quantifies all of the elements associated with the target cell. Thus, the measured quotas include both intracellular and extracellular elements (although our results indicate that extracellular elements were not a major fraction of quotas—see discussion below). While extracellular elements (including abiotically scavenged elements) may or may not be relevant for algal physiology, both fractions should be considered from a biogeochemical perspective. Extracellular metals will be ingested with cells when they are grazed and will be exported with cells when they sink. Extracellular metals will also be included in bulk particle analyses.

SXRF data from both runs were aggregated for statistical analyses. In total 267 cells were analyzed, including 160 cells mounted on Au grids and 107 cells mounted on Al grids (Table 1). Cellular element concentrations (mol/L cell biovolume) and stoichiometries were log-transformed prior to statistical analysis to normalize the data and stabilize variance. Data were identified as outliers on the basis of poor fit to an ANCOVA model that included station number

(nominal classification), sampling depth (nominal classification), log cell biovolume, and cell type [diatom, autotrophic flagellate (Aflag), autotrophic picoplankton (Apico; cells < 2 μm)]. Outlier analysis is necessary to identify cells compromised by spectral interferences (particularly from neighboring grid bars) or poor physiological condition (e.g., empty diatom frustules). Model analyses were made using JMP software (v. 7.0.2, SAS Institute). Jackknifed studentized residuals of this model were calculated, and all observations with values > 3.5 were excluded from further analysis (Twining et al., 2011). After the outlying cells were removed, the model was refit and the process repeated iteratively until outliers were no longer identified. This threshold value was chosen to guarantee that overall the probability of incorrectly excluding a cell given a normal distribution was <10%. Using this approach fewer than 3 cells, representing less than 3% of the dataset, were removed from the dataset for each parameter.

The element stoichiometries of the plankton were compared among stations, depths and cell types using geometric least-square (LS) means calculated by the ANCOVA model. These correct for differences in cell volume between the cell groups, as well as station and depth effects. Least-square means were also calculated for each station or depth grouping (ML or DCM). These correct for variable numbers of each cell type at each station or depth grouping, providing a standardized point of comparison for examining spatial or depth trends in element stoichiometries.

Phytoplankton pigment concentrations were collected from the surface mixed layer and analyzed with HPLC by Joaquin Chaves (Ocean Biology and Biogeochemistry, NASA Goddard Space Flight Center). Unfortunately HPLC samples from the 2010 cruise were compromised by unanticipated thawing during storage, so data are only available for the 2011 cruise. Pigments have been grouped according to broad size/function categories (e.g., microplankton, nanoplankton, picoplankton) following Hooker et al. (2005).

3. RESULTS

3.1 Biogeography of phytoplankton community

The NAZT transect crossed several biogeographical provinces (Longhurst, 1998). Stations 2011-1 through 2011-3 were within the Northwest Atlantic Shelves Province, which extends from the continental shelf and slope to the edge of the Gulf Stream (Fig. 1A). Although phytoplankton production and biomass in these waters were well below summertime peaks at the time of sampling (Longhurst, 1998), chlorophyll concentrations were still 0.8-2.6 $\mu\text{g/L}$ at the three stations closest to North America, with microplankton species (defined as diatoms and dinoflagellates) comprising 30-80% of the community. Phytoplankton were distributed throughout the mixed layer at these stations (Fig. 2A). Discrete HPLC measurements of photosynthetic pigments in surface waters indicated highest chlorophyll at Station 3 (Fig. 1B), while CTD fluorometry indicated highest sub-surface chlorophyll concentrations at Stations 1 and 2 (Fig. 2A). HPLC pigment data also show lower concentrations below this surface layer, matching the CTD fluorometry (data not shown), and both datasets indicate elevated phytoplankton biomass at the 3 stations nearest shore. Stations 2011-5 through 2011-8 were within the Gulf Stream Province, while stations to the southeast of the Gulf Stream were within the North Atlantic Subtropical Gyre Province where surface chlorophyll concentrations dropped to <0.2 $\mu\text{g/L}$ (Fig. 1B). Phytoplankton biomass in the subtropical gyre was dominated by small phytoplankton, with nanoplankton species (prymnesiophytes, cryptophytes and chrysophytes)

comprising 40-60% of the community (Fig. 1B). Subtropical stations were also characterized by a subsurface deep chlorophyll maximum (DCM; 75-100 m) immediately below the mixed layer (Fig. 2A). East of station 2011-19, the transect moved into the North Atlantic Tropical Gyre, which extends to the Cape Verde Islands. At these stations picoplankton (cyanobacteria and Prochlorophytes) became the dominant class of phytoplankton (Fig. 1B). Eastward of the Cape Verde Islands, the Canary Coastal Province is a region of upwelling and high Saharan dust flux/deposition. Unfortunately pigment data is not available for stations east of the Cape Verde Islands, but CTD fluorometer (Fig. 2A) and ocean color measurements from MODIS (Fig. 1A) indicate that elevated Chl was not present until close to the coast at station 2010-9. Concentrations of particulate P in the mixed layer also suggest elevated biomass closest to the coast. Despite the relative proximity to land, the station nearest Africa (2010-9) had a low population of microplankton based on the cells present on SXRF grids. This was also observed in the biogenic silica (bSi) measurements of Lam et al. (2015), which show 5-fold lower bSi at 2010-9 than at stations near the North American shelf (Fig. 11H). Between stations 2010-7 and 2010-6 the transect moved back into subtropical gyre waters with oligotrophic conditions. At the station closest to Lisbon (2010-1) phytoplankton biomass increased again, and the DCM shoaled (Figs. 2B).

3.2 Total particulate element concentrations

3.2.1 Spatial gradients

Total concentrations of particulate elements in the surface waters were also generally elevated near continental margins, with stronger gradients observed in the more lithogenic elements (Fig. 3). At the station closest to Lisbon (2010-1), Al was 10-fold higher in the surface mixed layer than offshore at 2010-5, and Ti and Fe concentrations were 5-fold higher, while P decreased only 2-fold moving offshore over the same transect. Moving from the Mauritanian upwelling (2010-9) to offshore tropical waters (e.g., 2011-22), Al and Ti dropped 5-fold, and Fe and P dropped 3-fold (Fig. 3). Traversing from the North Atlantic slope (2011-1) towards Bermuda along line W (e.g., to 2011-8) resulted in ca. 5-fold decreases in Al and Ti and again smaller drops (3-fold) in Fe and P. The higher particulate concentrations at the basin edges are expected given the continental sources of the lithogenic elements and elevated chlorophyll near the North American and African margins (Fig. 1A).

Vertical gradients in total particulate element concentrations within the upper 150m of the water column were weaker than basin-wide horizontal gradients. Biogenic elements such as P, Cd, Cu and Co were consistently elevated in the upper 75 m (and upper 50 m near the margins; Fig. 4), matching general trends in chlorophyll fluorescence. However sub-surface deep chlorophyll maxima seen prominently in the fluorescence data at offshore stations were not matched by increased particulate P (ie, biomass), except at the BATS station (2011-10) and the oligotrophic end-member off Lisbon (2010-5).

Aeolian dust is an important source of TEIs (including particulate TEIs) to the North Atlantic (Mahowald et al., 2005). Previous studies have documented an increase in aeolian metal inputs south of 25°N (Barrett et al., 2012; Buck et al., 2010; Mahowald et al., 2009), and we also observed an increase in Al moving towards 20°N, although surface Al was more elevated closer to Africa. The NAZT transect did not sample the waters south of 10°N, where dust deposition is expected to be greatest in the late fall and winter due to the seasonal location of the intertropical

convergence zone (Pohl et al., 2011; Ussher et al., 2013). Measured Ti concentrations in the upper ocean (ca. 50-200 pM offshore from Africa in eastern N. Atlantic) agreed with previous measurements in this area (Dammshäuser et al., 2013).

3.2.2 Correlations between elements

Information regarding the associations of trace metals with either biogenic or lithogenic particles can be obtained from bivariate correlations with either P or Ti, respectively (Ohnemus & Lam, 2014). Total particulate Fe and Al concentrations in the upper 150 m were indeed highly correlated with Ti concentrations ($r^2=0.94-0.95$; Table 2), indicating the predominant association of these elements with lithogenic minerals, while coefficients of determination (r^2) for all other elements with Ti were below 0.40. Regressions of all data produce overall Fe/Al, Ti/Al, and Fe/Ti molar ratios of 0.23, 0.021, and 10.8, respectively. These are within 20% of average ratio measured in upper continental crust (Wedepohl, 1995) and measured in aerosol particles collected on the same cruise (Shelley et al., 2014). In contrast, Cd, Co and Ni coefficients of determination with P were 0.44-0.61, while Fe and Al correlations with P were much lower ($r^2=0.10$; Table 2). This matches a previous study that reported positive correlations between biogenic material (i.e., POC) and particulate Cd, Co and Ni, as well as Cu, Zn and Mn, in North Atlantic surface waters (Kuss & Kremling, 1999).

3.3 Spatial gradients and elemental ratios in labile particulate element concentrations

Labile biogenic particles comprise only part of the overall particle population, and so an acetic acid-hydroxylamine leach was used to isolate trace elements associated with biogenic and chemically-labile material such as oxyhydroxides. Most particulate P (80% on average; Rauschenberg & Twining, 2015) was labile to the leaching procedure, and concentrations of several bioactive metals were more closely correlated with P in the labile fraction than in total particulate material (Table 2). Variations in labile P explained 65-78% of the variations in Cd, Zn and Ni—all significant increases over correlations between total particulate concentrations. Due to our focus on biogenic metals in the particulate fraction, labile metals are henceforth presented normalized to P. Spatial gradients in P-normalized labile metal concentrations reveal both horizontal and vertical trends (Fig. 5). Fe/P was highly elevated off the coasts of North America and Portugal, suggesting inorganic Fe hydroxides in these waters. Leached samples are not available for stations 2010-9 to 2010-12 closest to Africa to determine if the same trend was present in these waters. Mn/P was particularly high off North America but less so near the eastern margins. Lam et al. (2015) also found Mn oxyhydroxide concentrations to be highest in the near-surface waters near North America, suggesting a source in the shallow shelf waters, but not in waters off Africa. In contrast, Cu/P, Ni/P and Co/P tended to be higher in the middle of the ocean basin. Metal/P ratios were generally similar in the ML and DCM samples at each station, but three ratios were significantly different between the two depths across the dataset: Cd/P and Mn/P were significantly higher in the DCM, while Cu/P was significantly higher in the mixed layer (Fig. 5). These differences may be caused by scavenging (perhaps in the case of Mn) or differential remineralization (in the case of Cu/P).

The lability of each element (as operationally defined with the leach) was generally consistent across the section. P, Cd, Zn, Co, V, Cu, Ni and Mn were >70% labile, and Fe, Al and Ti were <20% labile (Fig. 6; Rauschenberg & Twining, 2015). Statistically significant zonal gradients in lability (based on linear regression in JMP; $p<0.01$) were also observed for some elements.

Significantly higher fractions of Cd and Cu were found to be labile towards the east, although the change in Cu lability was more notable (shifting from 60% in the west to 80% in east) than the change in Cd lability (shifting from 92% in the east to 98% in west). In contrast, particulate Al, Fe, Co and V were each significantly *less* labile in the eastern part of the section. However, the gradients were not identical: Al lability was enhanced (30-40%) near North America, with <20% lability east of Bermuda; while V was >60% labile across most of the basin but more refractory (30-40%) near Portugal. The lability of the remaining elements (P, Mn, Zn, Ti and Ni) did not change significantly across the transect.

3.4 Cellular metal stoichiometries

3.4.1 Estimating cell biomass

The elemental stoichiometries of trace metals in marine plankton provide valuable insights into biological nutrient requirements. Trace elements are quantified using SXRF on a cellular basis (i.e., mol/cell), but due to significant differences in cell size (individual cell biovolumes spanned 3 orders of magnitude), it is most useful to consider cellular metal quotas normalized to cell biovolume or to an elemental proxy of cell biomass, such as C, N, P or S. Carbon and N are not directly measurable with SXRF, but C quotas can be calculated from measurements of cell dimensions and used in conjunction with direct SXRF measurements of cellular P and S (Table 3). Cell C/P ratios spanned 2 orders of magnitude, from 21 to 1955, with an overall geometric mean C/P of 152, similar to mean bulk C/P in the upper 300 m on the cruise (128 ± 48 ; Twining et al., 2014b). These values agree with C/P ratios (200-1500) determined in individual picoplankton from the North Atlantic by Grob et al. (2013). In contrast, the range of cell C/S and P/S ratios were somewhat smaller, with overall geometric mean ratios of $101 (\pm 7)$ and $0.64 (\pm 0.4)$, respectively. Carbon/sulfur ratios in marine plankton are typically around 100 mol/mol (Payne & Price, 1999; Twining et al., 2011; Twining et al., 2004a), in good agreement with the mean ratio found here in the North Atlantic. Therefore, the approximate 50% elevation and depression of average C/P and P/S, respectively, relative to published values, suggest that cells along the transect were poor in P (Martiny et al., 2013) and potentially P limited (Vidal et al., 2003).

3.4.2 Differences in biomass element quotas between groups

The cells were characterized by a number of different taxa and morphotypes (Fig. 7). Due to difficulty in determining exact taxonomic classifications from light microscopy of dry samples, we grouped cells into only 3 functional groups: diatoms, autotrophic flagellates, and autotrophic picoplankton. Stoichiometries of biomass elements C, P and S varied significantly between these functional groups ($p < 0.001$, based on the ANCOVA test described above). Non-diatoms were about 50% depleted in P, relative to Redfield, and diatoms contained on average only a tenth of the P expected from Redfield (Table 3). Based on the closer agreement of group C/S ratios with expectations (approx. 100 mol/mol), this likely results from a real depletion of P and not a gross overestimate of cellular C. In fact, elevated C/P are to be expected given the poor growth conditions during the time of sampling (November-December), and similarly high C/P have been measured in pico- and nanoplankton from the Atlantic (Grob et al., 2013) and in P-limited cultures (Bertilsson et al., 2003). The calculated elemental stoichiometries of trace metals will be affected by normalizing to P in these P-deplete plankton, but the TE/P ratios nevertheless reflect the actual cellular quotas exhibited by plankton during the seasonal and environmental conditions experienced in late fall in the North Atlantic.

3.4.3 Basin-averaged trace metal stoichiometries

Bioactive trace metals occur in plankton cells at approximately 1000-fold lower concentrations than the major biomass elements, based on cell biovolumes as determined by microscopy. Overall geometric mean Fe, Zn and Cu concentrations were between 200 and 550 μM , and cellular concentrations of Mn, Co and Ni were approximately 10-fold lower still (Fig. 8). These match the relative cellular abundances observed elsewhere (Twining & Baines, 2013). However, there were some notable differences compared to plankton from other ocean basins. Geometric mean cellular P, S, Mn, Co, Ni and Zn concentrations in North Atlantic plankton were fairly similar to those measured with identical techniques in the equatorial Pacific Ocean (Twining et al., 2011), but Fe quotas were more than 3-fold higher than in the Pacific, indicating fundamental differences in the biogeochemical cycling of this element in the two ocean basins.

As with the biomass elements, taxonomic group differences in trace metals were also tested with ANCOVA models (Table 4) using cell size (as log biovolume), cell type, station, and station depth as effects. Models were run with metals normalized to each biomass element independently (P, S, and C), and effects found to be significant ($p < 0.05$; Table 4) in stoichiometries using at least two of the three biomass elements are considered to be of note. Only Fe and Co quotas were found to vary significantly as a function of cell biovolume, after controlling for taxonomic classification. In the case of Fe, smaller cells had significantly higher quotas than larger cells. The lack of a significant effect in Fe/C may be an artifact of the C/biovolume equation used, in which cellular C increases with cell volume as a power function rather than linearly (Menden-Deuer & Lessard, 2000). Iron quotas were also inversely related to cell volume in the Southern Ocean (Twining et al., 2004b). In contrast, Co quotas were significantly positively related to cell volume. Like Fe, Co may be scavenged onto particle surfaces in the ocean (Lee & Tebo, 1994; Moffett & Ho, 1996), but it is also possible that the trend is an artifact of the close fluorescence signals of Fe and Co. The K_{β} fluorescence peak for Fe (7058 eV) closely overlaps with the Co K_{α} peak (6930 eV). Although our model attempts to correct for this with using tightly-constrained Fe K_{α} / K_{β} branching ratios, the overlap may introduce additional uncertainty into the Co measurements, as noted above.

Iron was the only metal to consistently occur at significantly different concentrations in the three plankton groups. Although significant differences between cell types were seen for other metal/P ratios (Table 4), these are likely driven by differences in P quotas between diatoms and non-diatoms, as noted above, rather than by differences in metal contents. Diatoms contained approximately twice as much Fe (normalized to C) as autotrophic flagellates and picoplankton (Table 5). In comparison, in the equatorial Pacific and Southern Ocean diatoms contained either similar or slightly lower Fe/C ratios than other co-occurring taxa (Twining et al., 2011; Twining et al., 2004b). The other significant difference observed in C-normalized metal quotas was for Cu, with diatom Cu/C approximately 7-fold lower than in the other groups. The diatom Cu/C is similar to published cultures and bulk field data (Bruland et al., 1991; Ho et al., 2003; Twining & Baines, 2013), suggesting that the non-diatom Cu quotas are anomalously high. As discussed above, Al grids were used to avoid previous Cu contamination problems from Au grids. However, the possibility of an unidentified artifact remains.

3.4.4 Differences in element quotas between depths

In addition to differences between taxonomic groups, there were also some significant differences between cells in the mixed layer and DCM at some stations (Table 4). Multiple depths were sampled only at three of the stations (2011-1, 2010-9, 2010-5). At 2011-1 (off North America), Fe quotas were similar at both depths, probably because of their close proximity (30 m and 58 m) and the relatively well-mixed water column (Fig. 2A). However at 2010-9 and 2010-5 (both on the eastern side of the Atlantic), Fe quotas were 2- to 3-fold higher in ML cells (Fig. 9A). In the case of Co, quotas were somewhat lower in ML cells at 2011-1 but significantly higher (2- to 4-fold) in ML cells from the eastern NAZT stations 2010-9 and 2010-5 (Fig. 9B). These differences may reflect more significant aeolian inputs to surface waters at the southeastern edge of the transect (Mahowald et al., 2005). Shelley et al. (2014) found Co/Al in aerosols near Africa (including at station 2010-9, where dust fluxes were much higher) to match ratios in upper continental cruise, but Co/Al was about 10-fold higher in marine particles dominant over 2010-5.

3.4.5 Differences between cell quotas across stations

Significant differences were observed between stations for C-normalized quotas of all 6 metals ($p < 0.002$; Table 4). Cobalt and Zn showed approximately 4-fold spatial variations, but longitudinal gradients were not apparent (Fig. 10). Remarkably, Ni and Cu quotas were 30- to 50-fold elevated in cells along the basin margins—particularly near Africa, and both were lowest towards the central part of the basin (2011-16 and 2011-20). The behavior of Ni and Cu quotas diverged somewhat at station 2010-5: Cu quotas were lower than at 2010-9 and similar to those in the western part of the basin, while Ni quotas were similar to those at 2010-9 and higher than in the western part of the basin. Iron and Mn presented a third distribution pattern, with both elevated on the western margin but not on the eastern edge of the basin. Lowest values of Fe/C and Mn/C were found at the three easternmost stations sampled for SXRF (2010-5, 2010-9, 2010-12). About 2-fold higher values of both quotas were observed in the western-central gyre stations (2010-99, 2011-16, 2010-10). At the westernmost station (2011-1) Fe/C was another 2-fold higher still while Mn/C was similar to the stations to the east.

3.5 Spatial gradients in bulk and single-cell metal/P ratios

Spatial trends in cellular metal quotas obtained through SXRF and measurements of labile bulk particles can be directly compared through P-normalized stoichiometries. Each of the metals presents a unique relationship (Fig. 11). Iron/P ratios in cells and labile particles both increased from the east to the west along the zonal transect. Bulk ratios were between 40% and 4-fold higher than SXRF ratios, with the largest differences occurring at the eastern stations. The highest bulk Fe/P were at the stations closest to Lisbon (2010-1 and 2010-2), but SXRF data are not available for these stations. Bulk and cellular Mn/P also increased towards the west, but the two ratios diverged significantly in their values. Bulk Mn/P was approximately 6-fold higher than cellular Mn/P in the middle of the basin (2011-16) but 43- and 84-fold higher than cellular Mn/P at the basin margins (2010-9 and 2011-1, respectively). These offsets indicate significant abiotic scavenging of Mn oxides on particles that is not matched by scavenging of Mn onto co-occurring cells. It is also worth noting the different behavior of bulk Mn/P towards the two margins. Bulk Mn/P increased significantly along Line W moving from BATS towards the North American shelf, while similar increases were not seen near Africa. Although data for labile particles are not available from the stations closest to Africa (2010-9 to 2010-12), total particulate Mn/P (which are very similar to labile Mn/P due to the high lability of both elements

(Rauschenberg & Twining, 2015)) were also 10-20 mmol/mol and did not show an increase near the shelf.

Like Fe and Mn, bulk Co/P, Ni/P, Cu/P, and Zn/P ratios increased in the middle of the basin to various degrees, but unlike Fe and Mn these metals were not notably higher near the margins (Fig. 11). This clear trend is not seen in cellular Co/P, although the two Co measurements were generally within a factor of 2. In the case of Ni, cellular quotas actually showed a clear minimum in the middle of the basin, in stark contrast to the bulk data, such that the two measurements are within 40% of each other at the margins (2011-1 and 2010-5) but differ by 20- to 40-fold at the central stations (2011-16 and 2011-20). In the case of Cu, cellular and bulk data agree most closely in the middle of the basin (e.g., within 15% at 2011-16) and less so at the margins. Zinc data are characterized by more variability in both measurements, such that clear spatial trends are not evident. However labile and cellular Zn measurements were generally within a factor of 2 of each other, indicating that abiotic particles did not contribute significantly to the bulk ratios. Labile Cd/P ratios were relatively constant at 0.1-0.2 mmol/mol across the basin, but SXRF data are not available for comparison.

4. DISCUSSION

These data comprise the first basin-scale picture of phytoplankton metal quotas in the Atlantic Ocean. Data for individual cells at a subset of stations and depths can be extended to the broader particle population and geographic expanse through the data on labile particulate metal concentrations collected concurrently. The data provide opportunities for comparison at different levels, and we explore several of these below: basin-wide averages are compared to data from the Pacific and Southern Oceans; divergences between SXRF and ICPMS data are examined; and the role of the phytoplankton in metal partitioning and vertical distributions of dissolved metals are discussed. Relationships between metal quotas and horizontal gradients in dissolved and particulate metal inputs will be the focus of a separate paper.

4.1 Comparison of data to previous studies in the North Atlantic Ocean

Particulate trace element concentrations in the ML are generally in agreement with those measured previously (Barrett et al., 2012; Kuss & Kremling, 1999; Pohl et al., 2011), as well as those measured on the same cruise with in situ pumping (Twining et al., 2014b). Barrett et al. (2012) report particulate TE in the upper water column of the eastern North Atlantic Ocean along a N-S transect between 20°W and 30°W (CLIVAR section A16N), and we observed similar Fe (1-1.5 nM) in surface waters to the west of the Cape Verde Islands. Additionally, the enrichment in surface particulate Fe reported by Barrett et al. (2012) at 20°N, relative to 30°N, was also seen in our dataset during the meridional transect between stations 2010-5 to 2010-8. Particulate Al concentrations measured in this study also matched the general trends presented by Barrett et al. (2012). Overall basin-averaged concentrations agree within 2-fold with the average surface particulate concentrations reported by Kuss and Kremling (1999) except for Cd and Cu. For these two metals our mean values are about an order of magnitude lower. There is also generally good agreement in concentrations and spatial distributions of particulate trace elements measured with GO-FLO bottle sampling and in situ pumping conducted on the same cruise (Ohnemus & Lam, 2014; Twining et al., 2014b). Both approaches show lithogenics (Ti, Al, Fe) to be more enriched in ML water near Lisbon and North America than off Africa, where these particles are found lower in the water column.

Cellular SXRF measurements for *Synechococcus* picoautotrophs from mesoscale eddies near the BATS station have been reported previously (Twining et al., 2010). Basin-averaged APico Mn/P, Fe/P and Zn/P ratios presented here (Table 5) are within 25% of ratios in *Synechococcus* collected from a cyclonic eddy, while NAZT Ni/P ratios were slightly less than half of ratios in cyclonic eddy cells but similar to those of mode-water cells. For Mn, Fe, Ni and Zn the geometric LS mean stoichiometries fell within the range of eddy-specific average quotas measured by Twining et al. (2010).

4.2 Comparison of quotas to phytoplankton in other ocean basins and laboratory cultures

The overall geometric LS mean quotas for plankton in the North Atlantic are quite similar to those of plankton in the Pacific and Southern Oceans (Twining et al., 2011; Twining et al., 2004a; Twining et al., 2004b), with the exception of Fe quotas, which are about 3-fold higher than those in the Pacific. The higher Fe quotas likely result from higher dust—and perhaps shelf—inputs to the North Atlantic and consequently higher dissolved Fe concentrations compared to the Pacific and Southern Oceans (Hatta et al., 2014; Moore & Braucher, 2008). Higher Fe quotas have been simulated by ocean biogeochemical models (Moore et al., 2004) and suggested by water column dissolved metal concentration remineralization ratios (Twining & Baines, 2013), but previous measurements of euphotic zone particles have not revealed clear ocean basin differences (Twining & Baines, 2013). Thus, this is the first unequivocal indication of consistently elevated Fe quotas in North Atlantic plankton.

Measured Fe quotas are similar to or somewhat higher than those simulated in current biogeochemical models for the North Atlantic. The original version of the BEC model indicated quotas of only 6-8 $\mu\text{mol/mol C}$ (Moore et al., 2002), as suggested from measurements of remineralization ratios (Sunda, 1997; but see scavenging discussion below). But more recent models provide for higher quotas: the CESM model reports diatom Fe/C of 15-20 $\mu\text{mol/mol}$ in the North Atlantic (K. Moore, pers. comm.), and the PISCES reports Fe/C of 15-40 $\mu\text{mol/mol}$ for diatoms and 25-40 $\mu\text{mol/mol}$ for nanoplankton (A. Tagliabue, pers. comm.). The measured quotas also match phytoplankton Fe/C estimated from a deep-water remineralization model developed for the North Atlantic by Wu and Boyle (2002) (23-70 $\mu\text{mol/mol}$). The more significant difference with models is in the simulated spatial variations of the quotas. The PISCES model shows higher quotas toward the southeastern edge of the North Atlantic basin, likely due to higher dust inputs in this area. In contrast, measured cellular Fe quotas were not elevated close to Africa. This suggests that the aeolian inputs, which are highest in this region, may have quite limited availability and/or a short residence time in the upper water column, a point also noted by Ohnemus and Lam (2014).

Although somewhat higher than simulated in models, our measured Fe quotas are in-line with laboratory studies, which report diatom quotas to vary by approximately 200-fold over the range of Fe concentrations found in the ocean (Twining & Baines, 2013; assuming total dissolved Fe is equivalent to Fe' in EDTA-buffered media). At the concentrations measured in NAZT surface waters (Hatta et al., 2014), culture studies report diatom Fe/C of approximately 30-60 $\mu\text{mol/mol}$ (Sunda & Huntsman, 1995a; Twining & Baines, 2013). The agreement between measurements and cultures indicates that the assumption of full bioavailability of ambient dissolved Fe (despite being largely bound to strong organic ligands; (Buck et al., 2014)) is reasonably accurate. It is

also worth noting that culture studies have found considerable intra-species variability in metal quotas. For example, using a constant inorganic dissolved Fe concentration of 0.2 nM, Ho et al. (2003) found a mean Fe/C of 47 $\mu\text{mol/mol}$ for 4 diatom strains, but the quotas ranged from 9-89 $\mu\text{mol/mol}$. Our data included several different morphotypes of diatoms (Fig. 7), and the range of geometric LS mean Fe/C ratios observed across the basin (18-92 $\mu\text{mol/mol}$) matches the inter-taxa variability at just one environmental condition in the laboratory. This suggests that competition may select for a more narrow range of cellular TE stoichiometries in the natural systems.

Our data can also be compared to previous bulk measurements of plankton biomass. In the North Atlantic, Kuss and Kremling (1999) measured particulate metal concentrations of primarily-biogenic surface samples and suggested an elemental stoichiometry for North Atlantic plankton. Compared to this, our labile metal/P ratios are similar (within a factor of 2) for Zn, Ni and Co, however we measured lower Cd, higher Cu, and significantly higher Mn and Fe. More than half of our bulk samples from the upper 150 m had Fe/P between 12 and 22 mmol/mol, compared to an average of 5 mmol/mol reported by Kuss and Kremling (1999). The difference may be due to the exclusion by Kuss and Kremling (1999) of samples containing labile abiotic particles (e.g., Fe oxyhydroxides) from the averages for 'biogenic' samples. For example, they only included samples collected north of 45°, where dust inputs are much lower, thus missing some waters with higher Fe inputs. Our measurements of metal/P in particles and cells can also be compared to the canonical averages for Pacific and Southern Ocean plankton measured by Collier and Edmond (1984) and Martin and Knauer (1973) and summarized by Bruland et al. (1991). Our measured cell and bulk quotas span the Bruland et al. (1991) averages for Co, Ni and Zn (Figs. 10, 11). North Atlantic values are consistently higher for Cu across the basin, while Mn and Fe are higher mostly in the central and western parts of the basin, likely due to dust and shelf inputs. These comparisons highlight the unique value of the SXRF approach. In bulk studies, samples with lithogenic particles and those collected from areas with abiotic inputs are typically excluded, so that information about cellular responses to these same inputs is precluded.

4.3 Importance of phytoplankton to particulate metal pools

One of the reasons for interest in the metal contents of phytoplankton is their role in mediating the vertical distributions of dissolved metals. Vertical profiles of most bioactive metals show depletion in the euphotic zone and enrichment at depth due to uptake and remineralization from plankton (Bruland & Lohan, 2003). But as discussed above, there are other sources of particles to the surface ocean, and it is useful to consider the balance between abiotic particles (including lithogenic and authigenic minerals and detritus) and plankton. In remote regions with low lithogenic inputs, phytoplankton are thought to comprise a significant portion of particulate Fe (Coale et al., 2005; Frew et al., 2006), but this assumption is rarely directly tested, and estimates are even rarer in regions with notable lithogenic inputs such as the North Atlantic. Two approaches may be taken to estimate the fraction of particulate metals comprised of plankton. The simplest is to examine the fraction of particulate material determined to be labile by the Berger et al. (2008) rinse, although this will overestimate biogenic Fe and Mn due to inclusion of labile oxides. This approach predicts Cd, Zn, Co, Mn to be nearly entirely associated with biota, and Ni and Cu somewhat less so (60-80% in some parts of transect). Labile Fe comprises only 20-40% of total Fe (Rauschenberg & Twining, 2015).

Another approach is to use bulk labile P measurements to scale the SXRF measurements of biogenic metal/P ratios, and then compare these estimates of total biogenic metals to total particulate concentrations. This approach indicates far more variability in the biogenic fraction (Fig. 12). Cobalt and Zn are predicted to be largely biogenic, as expected. Particulate Ni is predicted to be entirely biogenic in the eastern portion of transect, and nearly so at the westernmost stations, but only 10% biogenic in the middle of the basin. Copper is characterized by similar trends, although in the case of Cu 100% of particles are biogenic in the middle of the basin while at the margin stations SXRF-based estimates significantly exceed measured labile particulate concentrations. These comparisons suggest that some of the regional SXRF values may not be good approximations of the community average. For example, average station-specific SXRF Ni/P ratios vary 8-fold across the transect, and using the higher margin quotas for calculations in the middle of the basin (where lower Ni/P quotas were actually measured) generates biogenic concentrations that will generally match bulk Ni measurements. Similarly, use of the 4-fold lower Cu/P ratios measured in the middle of the basin eliminates predictions of a biogenic fraction that exceeds total particulate Cu at certain stations. Less than 30% of particulate Mn and Fe are predicted to be biogenic. For Fe, this matches the Berger et al. (2008) leach results fairly well. In the case of Mn, we know that much of the particulate Mn is in the form of oxides; this comparison suggests that oxides comprise more than 60% of the labile particulate Mn (which is nearly equivalent to total particulate Mn) at all stations, and oxides are more than 90% of particulate Mn at some stations. The availabilities of these abiotic particulate TE fractions remain largely unconstrained.

Dissolved Al distributions are also thought to be partially controlled by associations with phytoplankton. Both field and laboratory studies indicate that Al is scavenged onto biogenic opal produced by diatoms (Gehlen et al., 2002; Orians & Bruland, 1986; Stoffyn & Mackenzie, 1982), and dissolved Al and silicate are highly anticorrelated across the western Atlantic Ocean (van Hulst et al., 2014). While total particulate Al and P were poorly correlated ($r^2=0.10$; Table 2), concentrations of labile particulate Al and P were rather well correlated ($r^2=0.73$), with labile P actually more closely correlated with labile Al than with labile Zn. This observation supports the contention that Al is scavenged onto phytoplankton biomass. To further investigate this, we examined correlations of labile particulate Al with biogenic silica concentrations measured by Lam et al. (2015) on in situ pump samples collected on the NAZT cruises. Biogenic silica was particularly elevated in the upper water column (<50 m) near the western North Atlantic margin (Fig. 11H) where microplankton such as diatoms were more abundant—although diatom biomass was still quite low given the time of year (Fig. 2B), and labile particulate Al and total biogenic were significantly correlated across the entire basin ($r^2=0.48$, $p=0.0005$; data not shown), based on 21 observations made at matching depths. Excluding one outlying observation improved the coefficient of determination (r^2) for labile particulate Al and biogenic silica to 0.89. This further supports the contention that Al is scavenged onto biogenic silica. This mechanism could help explain the increased proportion of labile particulate Al on the western edge of the transect (Fig. 6), where biogenic silica abundances were highest in the upper water column (Lam et al., 2015) (Fig. 11H). Based on 10-20% lability of particulate Al in the middle of the transect, it appears that the 30-40% lability seen on the western edge could be caused by approximately 20% of the particulate Al being adsorbed to biogenic material, including silica. However, we note that it is also possible that particulate lithogenic Al—some fraction of which is labile—was

simply correlated with biogenic silica, without Al being directly adsorbed to biogenic silica. Both may have been captured on the more highly loaded filters near the western margin. Thus, causation cannot be proved from correlation alone.

For the metals which appear to be largely abiotic, the potential role of detritus should also be considered. King et al. (2012) reported discrepancies between bulk and cell-specific Fe quotas and concluded that detrital particles likely contribute to these. Collier and Edmond (1984) noted that the Ni/P of material settling to deep ocean must be 2- to 4-fold higher than measured plankton values (based on their own bulk data), and a recent comparison of bulk and single-cell Ni/P ratios in sediment traps found exactly that: bulk Ni/P was 4- to 5-fold higher than in co-occurring sinking cells (Twining et al., 2014a). If our measured SXRF Ni/P ratios do represent the ambient community, then this study provides another indication of a significant and somewhat surprising role for non-cellular particles in Ni cycling. Some of this particulate Ni pool may come from anthropogenic emissions via oil combustion (Pacyna & Pacyna, 2001).

4.4 Cellular copper quotas

Previous SXRF studies have used Au electron microscopy grids that contain significant Cu and thus haven't reported Cu quotas. In this study we used Al grids at a subset of stations in order to avoid Cu contamination problems (although Al grids are inappropriate for Mn, Fe or Zn measurements due to high levels of these impurities). Copper is an essential metal for electron transport and other redox processes in cells. Dissolved Cu concentrations are rarely drawn down to sub-nanomolar levels in surface waters, but Cu largely occurs bound to strong ligands, and it has been suggested that low concentrations of unbound free Cu^{2+} may limit ammonia oxidizing archaea in certain situations (Jacquot et al., 2014). In other settings Cu may be toxic if elevated free Cu^{2+} concentrations are present (Mann et al., 2002). Phytoplankton Cu quotas have been estimated from remineralization ratios (Sunda & Huntsman, 1995b) and radioisotope uptake incubations (Semeniuk et al., 2008) but haven't been directly measured by SXRF before.

The measured Cu/C ratios match published quotas for diatoms, averaging ca. 4 $\mu\text{mol/mol}$ (Annett et al., 2008; Semeniuk et al., 2008; Sunda & Huntsman, 1995b), but flagellate Cu/C quotas and mean Cu/P ratios for all three groups are elevated relative to cultures (Guo et al., 2012). SXRF Cu/P ratios are comparable to bulk labile ICPMS Cu/P ratios in the middle of the basin, and the elevated Cu quotas at stations approaching the continental margins may be expected to result from anthropogenic inputs (Hong et al., 1996; Jordi et al., 2012; Paytan et al., 2009). The consistent spatial trends in Cu quotas also suggest that the measured quotas are not an artifact of the measurement technique. Although Cu toxicity may be controlled by the concentration of the free ion (Sunda & Guillard, 1976), uptake experiments with natural communities indicate that other dissolved species are also available (Semeniuk et al., 2008), so it is not straightforward to predict Cu quotas from dissolved Cu measurements in the North Atlantic.

4.5 Taxonomic variability in metal quotas

What are the ecological and biogeochemical implications of the observed taxonomic variability in plankton metal quotas? Only Fe quotas varied significantly between groups across all three biomass proxies, but Mn/P, Co/P and Zn/P were also significantly different between groups. In these cases diatom ratios were about 10-fold higher than ratios in non-diatoms. In no cases were

significant differences observed between autotrophic flagellates and smaller picophytoplankton. Biogeochemically, taxonomic differences could lead to altered metal cycling in situations where diatoms comprise a significant fraction of the phytoplankton community. Since diatoms were less than 10% of the community at all stations beyond the three nearest North America (Fig. 1B, though note that pigment data are not available for four stations closest to Africa), taxonomic differences are unlikely to explain spatial variations in bulk biogenic metal/P ratios (i.e., Fig. 5). However the higher diatom metal/P ratios may be important for vertical fluxes if diatoms are over-represented in export fluxes (Buesseler, 1998).

Taxonomic differences in metal quotas may be more informative about biological process and metal cycling. Previous SXRF studies in the Southern Ocean (Twining et al., 2004a) and equatorial Pacific (Twining et al., 2011) have found significant differences in the Mn, Co, Ni and Zn quotas of diatoms and co-occurring flagellate autotrophs, based on using S and C as biomass proxies. Since we see such differences only for Fe in the North Atlantic (discounting differences in P-normalized ratios as relating to P content rather than metal content), this suggests various North Atlantic cell-types are responding differently to their nutrient environments. The higher Fe quotas of diatoms also stand in contrast with previous field and laboratory work indicating lower diatom quotas. Since overall cellular concentrations of the non-Fe metals are similar between ocean basins (Fig. 8), basin-to-basin differences are not likely caused by different ambient metal concentrations. But there may be biological explanations. Diatoms contained significantly more Zn and Ni than flagellates in the other ocean basins. The absence of this difference in the North Atlantic may indicate a different taxonomic response to the lower N and P concentrations there. It has been suggested that picoautotrophs may increase their Ni and Zn quotas through up-regulation of metalloproteins such as alkaline phosphatase in response to lower N and P (Twining et al., 2010), and alkaline phosphatase activity was observed during the NAZT cruises (Wurl et al., 2013), as well as previously in western North Atlantic diatoms (Lomas et al., 2004). Since diatoms comprise a much smaller component of the phytoplankton community and were generally not observed in the oligotrophic ocean, it seems that this group is unable to compete in the oligotrophic waters, potentially due to differential physiological abilities that may be reflected in their elemental composition. More work, including direct protein measurements and nutrient-addition experiments, is required to address relationships between macronutrient availability and metal quotas in North Atlantic plankton.

Significant differences were observed between stations for all metal quotas, and these indicate changing metal availabilities. Correlations between cellular quotas and ambient metal inputs and concentrations will be examined quantitatively in a separate paper, but we note here the behaviors of cellular Fe, Ni and Cu. It was a surprise to observe 4-fold higher Fe quotas on the western side of the basin than on the eastern side, given the significance of African dust as a source of Fe to the Atlantic (Conway & John, 2014; Mahowald et al., 2005). Iron in aeolian dust appears to be less available than Fe from non-reductive shelf sediments. Supporting this, a smaller fraction of particulate Fe was chemically labile on the eastern side of the section (Fig. 6). Nickel and Cu quotas were both highest toward the margins, in particular the eastern margin. Both metals can come from anthropogenic emissions (Desboeufs et al., 2005; Jordi et al., 2012), which are likely to have stronger European than American inputs based on the prevailing winds at these latitudes (Paytan et al., 2009). These differences highlight the error in assuming a constant metal quota for plankton, even within a single ocean basin.

4.6 Does abiotic scavenging affect the metal quotas of North Atlantic plankton?

Several bioactive transition metals—most notably Fe—are highly particle-reactive in seawater and prone to scavenging (Goldberg, 1954). Indeed, dissolved Fe concentrations generally decrease along the path of thermohaline circulation (Parekh et al., 2005), and particulate Mn concentrations increase with depth as Mn is oxidized (Moffett, 1997; Sherrell & Boyle, 1992). Accordingly, chemical washes have been developed to remove externally-sorbed metals from plankton prior to analysis (Hassler & Schoemann, 2009; Tovar-Sanchez et al., 2003). However it remains challenging to study the process of scavenging in the ocean. Different types of particles are likely to have unique scavenging behavior, but these cannot typically be resolved with bulk particle measurements. Single-cell SXRF data informs our understanding of the importance of scavenging to metal quotas in the ocean.

Bulk metal/P ratios were similar to cellular metal/P ratios for some elements (Co, Zn) but were systematically higher for Fe and Mn (Fig. 11). While these metals *are* most prone to scavenging, the comparison of bulk and cellular data may be driven by inclusion of detrital, authigenic and lithogenic particles on the filters rather than by direct scavenging onto cells. Treatment of particles with an oxalate wash reduced bulk Fe/P ratios, but they remained higher than ratios in co-occurring cells (Rauschenberg & Twining, 2015). In fact, two-dimensional maps of Fe distributions in diatoms (which had the highest Fe quotas) indicate that scavenged Fe did not comprise a significant portion of the Fe associated with these cells (Fig. 13). Scavenged or adsorbed Fe would be either evenly distributed over the outside of the cells (indicated via the frustules in the Si maps) or highly localized in ‘hotspots’ corresponding to adsorbed particles. There are some cells with features that approximate this, but these hotspots do not contribute significantly to the cellular Fe contents (<25%; Twining & Nuester, 2012). Rather, in most cells Fe was concentrated in the central biomass-rich region of the cell. This suggests that there is not significant scavenging of Fe onto live plankton cells, even in Fe-rich waters such as the North Atlantic. This is consistent with the presence of excess dissolved Fe-binding ligands (Buck et al., 2014). On the other hand, cellular Fe and Co quotas were significantly negatively related to cell volume, indicating that smaller cells had higher quotas (after controlling for taxonomy). This could be the result of some scavenging, or alternatively be driving by surface-associated metals in the smaller cells with higher surface area/volume ratios.

Although scavenging onto the surfaces of living phytoplankton cells does not appear to be significant for Fe, scavenging is likely to be important deeper in the water column (Boyd & Ellwood, 2010). Previous studies have used stoichiometries of dissolved Fe, N, P and/or dissolved oxygen in the water column to estimate the Fe/C ratios of sinking biomass (Sunda, 1997), in the absence of cell-specific measurements. In the Atlantic Ocean this approach has produced Fe/C estimates of approximately 5-12 $\mu\text{mol/mol}$ (Bergquist & Boyle, 2006; Fitzsimmons et al., 2013; Rijkenberg et al., 2012; Sunda, 1997; Ussher et al., 2013), including data from the NAZT cruise (Hatta et al., 2014) (Table 6). These ratios are several-fold higher than measured with the same approach in the low-Fe Pacific Ocean (Sunda, 1997), but they are at least 3-fold below Fe/C measured in cells in this study (Table 6). Similarly, cellular Fe/P calculated from subsurface remineralization of these two cellular constituents in the Atlantic range from 0.2-0.4 mmol/mol (Hatta et al., 2014; Rijkenberg et al., 2014), while our

measurements, as well as previous bulk estimates of plankton Fe/P in the Atlantic (Kuss & Kremling, 1999), are an order of magnitude higher at 2-12 mmol/mol.

This discrepancy results from Fe scavenging and differential remineralization of cellular nutrients in the water column. Although remineralization rates exceed scavenging rates in the upper 1,000 m of the ocean (Boyd & Ellwood, 2010), scavenging still occurs at these depths, and the resulting dissolved stoichiometries are actually *net* remineralization ratios that can be significantly lower than ratios in sinking biogenic material. Hatta et al. (2014) estimate that 60-90% of remineralized Fe may be lost to scavenging. Differences are likely to be greater in ocean regions with more particle inputs and fluxes, such as the North Atlantic Ocean (Ohnemus & Lam, 2014). Iron may also be lost more slowly from sinking cells than other nutrients such as P (Twining et al., 2014a), further complicating the use of dissolved ratios to infer cellular ratios. Our measurements confirm that water column nutrient ratios underestimate plankton Fe stoichiometries.

Dissolved Fe/P ratios from the water column have been used in calculations of Fe* ($\text{Fe}^* = [\text{DFe}] - R_{\text{Fe/P}} \times [\text{PO}_4^{3-}]$), a proxy for ‘excess’ dissolved Fe in the water column (Parekh et al., 2005). Rijkenberg et al. (2014) use Fe/P of 0.33 mmol/mol in their calculations, based on their measurements of DFe and P in the OMZ, and a previous review reported average dissolved Fe/P of 1.1 ± 0.4 mmol/mol for the North Atlantic (Twining & Baines, 2013). In contrast, we measured average Fe/P of 5 mmol/mol in cells. Rijkenberg et al. (2014) use their Fe* calculations to conclude that dissolved Fe concentrations in the western Atlantic Ocean are limiting only poleward of approximately 50°N or 50°S. Use of our higher Fe/P for Fe* would significantly decrease the predicted area of Fe-replete waters. However, the measured Fe quotas are notably above those typically of Fe-limited phytoplankton (Sunda et al., 1991) and likely represent some level of luxury uptake. Therefore, it may be appropriate to use a Fe/P ratio of ca. 0.5 mmol/mol, which is also close to that measured for Fe-limited phytoplankton (Marchetti et al., 2006; Sunda & Huntsman, 1995a; Twining & Baines, 2013).

To first order, elemental correlations are useful indicators of the associations of trace metals with either lithogenic or biogenic particulate matter (or both). But they also demonstrate the limitation of using static stoichiometries of end-member particle types to estimate particle composition. For example, the distributions of Cd and P in the ocean are known to be closely related (Boyle et al., 1976; de Baar et al., 1994), however total particulate P concentrations explain only 49% of the variability in total particulate Cd concentrations in the surface ocean of the North Atlantic, and only 17% of total particulate Zn variability is explained by P (Table 2). These perhaps surprisingly weak bivariate correlations likely result from a combination of associations of metals with non-biogenic (likely detrital and lithogenic) material, differential elemental remineralization of elements from particles, and dynamic metal/P stoichiometries in plankton. For example, Barrett et al. (2012) concluded that anthropogenic emissions were an important source of particulate Zn, but Zn is often considered an element primarily associated with plankton biomass (Collier & Edmond, 1984).

5. CONCLUSIONS

This study provides the first reported single-cell phytoplankton metal quotas from across the North Atlantic Ocean, a basin characterized by significant aeolian inputs from North Africa and

anthropogenic inputs from North America and Europe. Cellular metal quotas are shown to be broadly similar to those in other ocean basins with the exception of Fe, which occurred at approximately 3-fold higher levels in North Atlantic cells. Quotas showed different spatial trends, with Fe and Mn quotas elevated in cells near North America, and Ni and Cu quotas elevated in cells towards both eastern and western margins of the basin. Comparisons of cell quotas with bulk labile particulate ratios show general agreement but also some systematic differences (e.g., in Fe/P, Mn/P, and Ni/P) that indicate significant scavenging or detrital components in the labile particulate pool.

Metal quotas were not consistently statistically different between the three taxonomic groupings except for Fe, which was higher in diatoms. This highlights the generally more plastic nature of diatoms, due to their nutrient storage capabilities (Marchetti et al., 2009). All taxa were generally P-deplete across the section, although this effect was strongest in the diatoms, and this likely elevates the P-normalized quotas further above those observed in other regions and in culture. Phytoplankton comprise a consistently significant portion of particulate Co, Ni, Cu, and Zn in the upper ocean, but they are less than 50% – and often less than 20% – of particulate Fe and Mn – in the North Atlantic.

Questions remain regarding metal accumulation and cycling by plankton in the North Atlantic Ocean. Prokaryotic organisms such as *Prochlorococcus* and heterotrophic bacteria are a major component of biomass in the gyre (Buck et al., 1996) but were not included here due to methodological limitations. Future work could focus on metal accumulation and cycling by these groups, which are particularly under-represented in metal quota culturing experiments. The timing of sampling (late fall/early winter) was also not ideal since most phytoplankton populations are in decline and likely experiencing light limitation and/or nutrient stress. Finally, the importance of scavenging of metals onto phytoplankton, and the roles of phytoplankton vs. lithogenic and detrital particulate fractions (including scavenged metals) in metal export and vertical distributions, are still poorly understood. Given the unprecedented rich dataset produced by the GEOTRACES program in the North Atlantic, future papers and synthesis should move beyond distributions and examine rate and flux processes.

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Table 1. Summary of SXRF samples collected during US GEOTRACES NAZT cruises. Cells were collected either from the mixed layer (ML; typically 25 m) or deep-chlorophyll maximum (DCM) using GO-FLO bottles, or from the surface (ca. 2 m) using a towed fish, onto either Au or Al TEM grids. Cells were categorized into three groups: diatoms, autotrophic flagellates (AFlag), and autotrophic picoplankton (APico; < 2 μ m). The unbalanced sampling design resulted from changing plankton communities across stations and generally low cell concentrations in late fall and early winter.

Cruise	Date	Station	Lat (°N)	Lon (°W)	Depth (m)	Au grids			Al grids			Total
						Diatoms	AFlag	APico	Diatoms	AFlag	APico	
2010	21 Oct 2010	5	31.0021	21.9999	ML (30)		12	2		7	1	22
					DCM (101)		13	1		7	1	22
	27 Oct 2010	9	17.3505	18.2545	ML (28)	2	15	2		6		25
					DCM (50)	1	12	7	1	12	1	34
	2 Nov 2010	12	17.3998	24.4998	DCM (73)		8	6		6		20
	10 Nov 2010	99	17.6857	31.0857	Surface (2)	5	11					16
2011	19 Nov 2010	153	21.5610	61.5580	Surface (2)	1	10					11
	7 Nov 2011	1	39.6840	69.8139	ML (30)	4	6					10
					DCM (58)	4	7	5	3	9		28
	19 Nov 2011	10	31.7534	64.1748	ML (40)				1	7		8
					DCM (96)	2	13			10		25
	23 Nov 2011	12			DCM (65)					9	4	13
	30 Nov 2011	16	26.1369	44.8262	ML (40)	1	9	1				11
					DCM (92)				1	10	2	13
	3 Dec	20			DCM (105)					8	1	9

2011

Total cells	20	116	24	6	91	10	267
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Table 2. Coefficients of determination (r^2) for bivariate linear regressions of trace metals against P or Ti, calculated using either total particulate concentrations in the upper 150m, or just labile particulate concentrations in the upper 150 m.

	Cd	Zn	Ni	Co	Cu	V	Mn	Fe	Al
<i>Total</i>									
Vs. P	0.49	0.17	0.44	0.61	0.28	0.23	0.17	0.10	0.10
Vs. Ti	0.02	0.35	0.26	0.25	0.14	0.09	0.23	0.95	0.94
<i>Labile</i>									
Vs. P	0.73	0.65	0.78	0.43	0.23	0.41	0.51	0.41	0.73
Vs. Ti	0.01	0.13	0.11	0.02	0.12	0.01	0.05	0.48	0.27

Table 3. Geometric least-square mean stoichiometries (± 1 SE) of biomass elements C, P and S in plankton cells from the North Atlantic Ocean.

	n	C:P	C:S	P:S
Diatom	20	1,229 $\pm$ 233	175 $\pm$ 21	0.14 $\pm$ 0.02
Aflag	116	140 $\pm$ 10	104 $\pm$ 5	0.70 $\pm$ 0.04
APico	24	153 $\pm$ 26	114 $\pm$ 14	0.79 $\pm$ 0.13

Table 4. Significance (P values) of various effects (log cell biovolume, cell type, station number, depth) on metal/biomass stoichiometries, as determined with ANCOVA tests. Tests were run separately for each metal normalized to one of three biomass elements (P, S, C). P values <0.05, denoting statistical significance, are in boldface. Cell S was not quantified in cells mounted on Al grids, precluding testing of effects on S-normalized Ni and Cu quotas. Multiple depths were not analyzed at all stations, so depth was nested within station as an effect.

	Mn	Fe	Co	Ni	Cu	Zn
<i>Log volume</i>						
<i>P</i>	0.0475	0.0011	0.0216	0.8822	0.0026	0.6237
<i>S</i>	0.6041	0.0077	0.0002	--	--	0.1038
<i>C</i>	0.6733	0.1382	<0.0001	0.4486	0.1629	0.0340
<i>Cell type</i>						
<i>P</i>	0.0003	<0.0001	<0.0001	0.6084	0.5789	<0.0001
<i>S</i>	0.6089	<0.0001	0.7259	--	--	0.5177
<i>C</i>	0.4647	0.0206	0.1106	0.4013	0.0040	0.2288
<i>Station</i>						
<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.0016	0.0001
<i>S</i>	0.0011	<0.0001	0.0401	--	--	0.0002
<i>C</i>	0.0772	<0.0001	0.0064	<0.0001	<0.0001	<0.0001
<i>Depth[Station]</i>						
<i>P</i>	0.0651	0.6429	0.0144	0.0082	0.0244	0.5364
<i>S</i>	0.0913	0.0001	<0.0001	--	--	0.1314
<i>C</i>	0.1328	0.0012	0.0006	0.1210	0.2776	0.8089

Table 5. Geometric least-square mean stoichiometries (± 1 SE) of trace metals in plankton cells from the North Atlantic Ocean.

	Mn	Fe	Co	Ni	Cu	Zn
<i>Normalized to P (mmol/mol)</i>						
Diatom	2.3 ± 0.9	76 ± 15	3.1 ± 1.2	0.45 ± 0.33	9.5 ± 5.8	12 ± 3
Aflag	0.44 ± 0.05	5.0 ± 0.4	0.14 ± 0.01	0.32 ± 0.05	5.3 ± 0.7	1.8 ± 0.2
APico	0.34 ± 0.09	5.0 ± 0.9	0.15 ± 0.04	0.48 ± 0.24	4.6 ± 1.8	1.2 ± 0.3
<i>Normalized to C (μmol/mol)</i>						
Diatom	2.3 ± 0.9	57 ± 9	0.42 ± 0.23	0.5 ± 0.5	4.2 ± 2.3	9 ± 2
Aflag	3.2 ± 0.4	35 ± 2	0.92 ± 0.11	1.6 ± 0.3	25 ± 3	13 ± 1
APico	2.4 ± 0.7	33 ± 6	1.5 ± 0.5	1.8 ± 1.0	33 ± 15	10 ± 3

Table 6. Comparison of Fe/P and Fe/C ratios in euphotic zone cells (measured with SXRF) and particulate matter (measured with ICPMS) to those determined from regressions of dissolved Fe vs. apparent oxygen utilization (AOU) or dissolved N or P. Dissolved data are from Hatta et al. (2014), for the upper 1,000 m. Regressions were performed with data collected from surface down to the depth of maximum dissolved Fe. Regression data were converted to Fe/C using a C/AOU ratio of 1.6 (Martin et al., 1987), a C/N ratio of 6.6 (Redfield, 1958), or a C/P ratio of 106 (Redfield, 1958).

Station	<u>Fe/P (mmol/mol)</u>			<u>Fe/C (μmol/mol)</u>			
	SXRF	ICPMS	Dissolved	SXRF	Dissolved (AOU)	Dissolved (P)	Dissolved (N)
2011-1	12-23	17-36	negative	79-94	negative	negative	negative
2011-10	8.3	6.8	0.36	35	2.7	3.4	3.6
2011-16	9.7	34.3	0.19	36	1.7	1.8	2.0
2010-12	5.9	10.9	0.74	15	4.6	7.0	6.2
2010-9	3.0-3.6	8-41	1.4	26-38	5.9	13	9.8
2010-5	2.6	10-14	0.38	15-38	2.7	3.6	3.6
Previous studies in eastern tropical North Atlantic Ocean							
Hatta et al. (2014)	--	--	--	--	6.6-7.4	--	--
Fitzsimmons et al. (2013)	--	--	--	--	9.6-12.4	--	--
Ussher et al. (2013)	--	--	--	--	ca. 1.5-13	--	--
Rijkenberg et al. (2012)	--	--	--	--	4.8 ± 3.3	--	--

Figure captions

Fig. 1. **A)** Map of stations sampled during two US GEOTRACES cruises across North Atlantic Ocean plotted over MODIS chlorophyll image averaged for November and December in 2010 and 2011. Images from each year showed no significant differences and have been averaged here. White lines indicate the approximate boundaries of biogeographical regions (Northwest Atlantic Shelves Province—NWCS; Gulf Stream Province—GFST; North Atlantic Subtropical Gyre—NSTG; North Atlantic Tropical Gyre—NATR; Canary Coastal Province—CNRY) in the Atlantic Ocean, as defined by Longhurst (1998). The 2010 cruise (squares) sampled the water column from Portugal to the Cape Verde Islands, and surface samples only were collected for SXRF at two stations from Cape Verde Islands to Charleston, SC. The 2011 cruise (diamonds) sampled the water column from Woods Hole, MA, to the Cape Verde Islands. Stations with SXRF samples are indicated with filled symbols. **B)** Fraction of total photosynthetic pigments representing picoplankton (pPF: zeaxanthin and total chlorophyll *b*), nanophytoplankton (nPF: 19'-hexanoyloxyfucoxanthin, 19'-butanoyloxyfucoxanthin, alloxanthin), and microphytoplankton (mPF: fucoxanthin and perdinin), as defined by Hooker et al. (2005). Also plotted as a function of station number are the total chlorophyll *a* concentrations. All pigment data are for surface water samples, as measured with high performance liquid chromatography.

Fig. 2. **A)** Depth profiles of chlorophyll *a*, mixed layer depth (black lines), and SXRF sampling depths (black circles) across the E/W portion of the transect. Chlorophyll fluorescence is from the GEOTRACES carousel fluorometer. Mixed layer depth was calculated from surface potential density + 0.125, as provided by Mariko Hatta (Univ. of Hawaii). Cruise year and station numbers are indicated above the section for SXRF stations. **B)** As for part A, but for the N/S portion of the transect. The section begins near Lisbon.

Fig. 3. Total particulate concentrations of the primarily-lithogenic elements Ti, Fe, and Al in the upper 150 m of the water column across the North Atlantic Ocean. The section runs from Woods Hole, MA, to Lisbon, with the turn at the African continent marked with a vertical black line. Note the color scales are not linear and have been scaled to delineate samples at both the upper and lower end of each scale. Outlying values are noted parentheses next to the data points.

Fig. 4. Total particulate concentrations of primarily-biogenic (P, Cd, Ni, Co, Cu, Zn) or labile (V, Mn) elements in the upper 150 m of the water column across the North Atlantic Ocean. The section runs from Woods Hole, MA, to Lisbon (as in figure 3), with the turn at the African continent marked with a vertical black line. Note the color scales are not linear and have been scaled to delineate samples at both the upper and lower end of each scale.

Fig. 5. P-normalized ratios of labile particulate Fe, Cu, Ni, Co, Cu, Zn, V and Mn (mmol/mol) in the upper 150 m of the water column across the North Atlantic Ocean. The section runs from Woods Hole, MA, to Lisbon (as in figure 3), with the turn at the African continent marked with a vertical black line. Note the color scales are not linear and have been scaled to delineate samples at both the upper and lower end of each scale.

Fig. 6. Percent of total particulate P, Ni, Cd, Cu, Mn, Fe, Co, Al, Zn, Ti, and V found to be labile using the leach of Berger et al. (2008). Data are for the upper 150 m of the water column across

the North Atlantic Ocean. The section runs from Woods Hole, MA, to Lisbon (as in figure 3), with the turn at the African continent marked with a vertical black line. The color scale is the same for all panels.

Fig. 7. Light micrographs and false-color SXRF maps of P, S, and Fe distributions in 5 cells from the North Atlantic Ocean, demonstrating the various cell morphologies: A) autotrophic picoplanker (Apico); B) diatom, similar to *Cylindrotheca closterium*; C) dinoflagellate (Aflag); D) diatom, similar to *Navicula* sp. or *Nitzschia bicaudata*; E) large two-lobed autotrophic flagellate or dinoflagellate (Aflag). Scale bar indicates 2 μm in panels A, C, D, and E, and scale bar indicates 10 μm in panel B.

Fig. 8. Overall geometric mean ($\pm$ SE) P, S, Mn, Fe, Co, Ni, Zn, and Cu concentrations in cells collected from the North Atlantic Ocean (NAZT; white bars) and in cells collected from the equatorial Pacific Ocean and published previously (EqPac; black bars; Twining et al., 2011).

Fig. 9. Ratio of Fe (A) or Co (B) quotas in cells collected from either the mixed layer (ML) or deep chlorophyll maximum (DCM) at three stations. Metal quotas are presented normalized both to C (black bars) and to S (white bars). Bars are ratios of geometric least-square means $\pm$ propagated SE. Dashed line indicates no difference in ratios in cells from ML and DCM.

Fig. 10. Carbon-normalized metal quotas for cells collected at 7 stations across the North Atlantic Ocean. Stations are ordered from west (left) to east (right); the vertical line indicates the location of the African continent along the transect. Symbols are geometric least-square means $\pm$ SE. Horizontal lines indicate the biogenic metal stoichiometries presented by Bruland et al. (1991). Three trends are apparent: elements with higher quotas in the west (Fe, Mn); elements with no clear spatial trend (Co, Zn); elements with higher quotas towards North America, Africa, and Europe (Ni, Cu).

Fig. 11. Phosphorus-normalized metal ratios for labile particulate matter (black symbols) or phytoplankton cells (white symbols) collected from the euphotic zone across the North Atlantic Ocean. Stations are ordered from west (left) to east (right); the vertical line indicates the location of the African continent along the transect. Symbols are geometric means $\pm$ SE for cells. For particles, symbols are means of metal/P ratios measured in mixed layer and DCM $\pm$ range/2. Horizontal lines indicate the biogenic metal stoichiometries presented by Bruland et al. (1991). The gray symbols in panel H are biogenic silica concentrations in the upper 50 m of the water column at each station as reported by Lam et al. (2015).

Fig. 12. Fractions of total particulate metal concentrations in the euphotic zone calculated to be contributed by phytoplankton. Biogenic particulate metal concentrations were calculated as the product of labile particulate P and cellular least-square mean metal/P ratios for phytoplankton from the nearest station from which SXRF samples were analyzed. Symbols are means $\pm$ range/2 for particulate matter collected from mixed layer and deep chlorophyll maximum at each station.

Fig. 13. SXRF maps of Si and Fe in 4 diatoms collected from stations 2010-99 (A, B) and 2011-1 (C, D). The color scales are different for each map and are intended only to show relative

spatial element distributions. For each cell, Si is associated with the frustule and is relatively evenly distributed across the cell. Iron is more localized in the central biomass portion of the cell. Scale bar is 2 μm .

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Figure 1

Figure 2A

Figure 2B

Figure 3

Figure 5

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Figure 7

Figure 8

Figure 9A

Figure 9B

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