J. Phycol. **39,** 1145–1159 (2003)

THE ELEMENTAL COMPOSITION OF SOME MARINE PHYTOPLANKTON[1]

Tung-Yuan Ho[2,3]

Department of Geosciences, Princeton University, Princeton, New Jersey 08544, USA

Antonietta Quigg,[3] *Zoe V. Finkel*

Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, New Jersey 08901, USA

Allen J. Milligan

Department of Geosciences, Princeton University, Princeton, New Jersey 08544, USA

Kevin Wyman

Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, New Jersey 08901, USA

Paul G. Falkowski

Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, New Jersey 08901, USA; and Department of Geology, Rutgers University, New Jersey 08901, USA

and

François M. M. Morel

Department of Geosciences, Princeton University, Princeton, New Jersey 08544, USA

We analyzed the cellular content of C, N, P, S, K, Mg, Ca, Sr, Fe, Mn, Zn, Cu, Co, Cd, and Mo in 15 marine eukaryotic phytoplankton species in culture representing the major marine phyla. All the organisms were grown under identical culture conditions, in a medium designed to allow rapid growth while minimizing precipitation of iron hydroxide. The cellular concentrations of all metals, phosphorus, and sulfur were determined by high-resolution inductively coupled plasma mass spectrometry (HR-ICPMS) and those of carbon and nitrogen by a carbon hydrogen nitrogen analyzer. Accuracy of the HR-ICPMS method was validated by comparison with data obtained with ^{55}Fe radioactive tracer and by a planktonic reference material. The cellular quotas (normalized to P) of trace metals and major cations in the biomass varied by a factor of about 20 among species (except for Cd, which varied over two orders of magnitude) compared with factors of 5 to 10 for major nutrients. Green algae had generally higher C, N, Fe, Zn, and Cu quotas and lower S, K, Ca, Sr, Mn, Co, and Cd quotas than coccolithophores and diatoms. Co and Cd quotas were also lower in diatoms than in coccolithophores. Although trace element quotas are influenced by a variety of growth conditions, a comparison of our results with published data suggests that the measured compositions reflect chiefly the intrinsic (i.e. genetically encoded) trace element physiology of the individual species. Published field data on the composition of the planktonic biomass fall within the range of laboratory values and are generally close to the approximate extended Redfield formula given by the average stoichiometry of our model species (excluding the hard parts):

$$(C_{124}N_{16}P_1S_{1.3}K_{1.7}Mg_{0.56}Ca_{0.5})_{1000}Sr_{5.0}Fe_{7.5}Zn_{0.80}Cu_{0.38}Co_{0.19}Cd_{0.21}Mo_{0.03}$$

While clearly this elemental stoichiometry varies between species and, potentially, in response to changes in the chemistry of seawater, it provides a basis for examining how phytoplankton influence the relative distributions of the ensemble of major and trace elements in the ocean.

Key index words: **culture medium; elemental composition; ICPMS; marine phytoplankton; nutrients; quotas; Redfield ratio; trace elements; trace metals; trace nutrients**

Abbreviation: **HR-ICPMS, high-resolution inductively coupled plasma mass spectrometry**

Over the past two decades, both culture and field studies have revealed that trace metals can be important in controlling primary production and regulating the community structure of marine phytoplankton. For

[1] Received 9 May 2003. Accepted 4 September 2003.

[2] Author for correspondence and present address: Institute of Earth Sciences, Academia Sinica, Taipei, Taiwan.
e-mail tyho@earth.sinica.edu.tw.

[3] T.-Y. Ho and A. Quigg contributed equally to this study.

example, on the basis of large- and small-scale field experiments, iron is now known to limit phytoplankton growth in some areas of the ocean (Martin and Fitzwater 1988, Price et al. 1991, Martin et al. 1994, Coale et al. 1996, Boyd et al. 2000). Culture studies have shown that several trace metals can limit the growth of model phytoplankton species at unchelated concentrations in the range estimated for surface seawater (Anderson et al. 1978, Brand et al. 1983, Morel et al. 1994, Sunda and Huntsman 1995a, b, Saito et al. 2002). The differential effects that are observed on various species imply that metals influence the physiology and ecology of marine primary producers.

Despite growing interest in the subject, there is limited information regarding the trace element content of marine phytoplankton. A number of publications provide cellular quotas for a few trace elements in some species (particularly the estuarine diatom *Thalassiosira weissflogii*) under specific culture conditions (Harrison and Morel 1986, Price and Morel 1990, Lee and Morel 1995). Sunda and Huntsman published detailed studies on the trace metal concentrations of a few model species, particularly the open ocean coccolithophore *Emiliania huxleyi* and the diatoms *Thalassiosira pseudonana* (neritic) and *Thalassiosira oceanica* (oceanic) in addition to *T. weissflogii* under a wide range of conditions (Sunda and Huntsman 1995b, 1998a, 2000). But there are no synoptic data for all the elements that are known to be used by phytoplankton and for representatives of major phytoplankton taxa. The most widely cited reference on the elemental composition of marine phytoplankton is based on a 30-year-old field study of the composition of suspended particulate matter collected by plankton tows in Monterey Bay (Martin and Knauer 1973).

There are two reasons for the paucity of such data. First, radiotracer experiments, usually carried out in the laboratory, do not allow more than a few metals to be measured simultaneously. Further, we know that the cellular concentration of a given element in phytoplankton is affected by its bioavailable concentration in the medium and also by the concentrations of major nutrients and other trace elements (Bruland et al. 1991). Hence, the very design of experiments to measure a representative trace element composition of phytoplankton is difficult.

Here we take advantage of the progress in analytical instrumentation and in our understanding of the trace metals physiology of marine phytoplankton to obtain systematic data on the composition of 15 marine species, 4 oceanic and 11 neritic, representing five eukaryotic phyla (Table 1). High-resolution inductively coupled plasma mass spectrometry (HR-ICPMS) provides a means for simultaneous and extremely sensitive multielemental analysis. In addition, previous studies show that cellular quotas for individual elements are normally regulated over a range of medium concentrations. This allows us to choose culture conditions that provide meaningful data while being mindful of the complex effects of the concentrations of major nutrients and other trace elements. We discuss the variations in elemental quotas among species and compare our data with previous detailed laboratory studies of a few individual species. A further comparison of the range and average of the average composition measured in the laboratory with available field data provides insight into the variations in phytoplankton composition in seawater.

MATERIALS AND METHODS

All apparatus for medium preparation, algal culturing and sampling, and elemental analysis was prepared according to rigorous acid cleaning procedures (Cullen and Sherrell 1999). Filters and filtration apparatus were precleaned with 10% trace

TABLE 1. Species, taxa, clones, specific growth rate $\rho(d^{-1})$, cellular volume $(\mu m)^3$, and maximum quantum yields of photosynthesis (F_v/F_m) for the marine phytoplankton measured in this study.

Species	Taxa	Clone[a]	Authority	Provenance	$\rho(d^{-1})$	Cellular volume $(\mu m)^3$	Fv/Fm
Dunaliella tertiolecta	Chlorophyceae	CCMP 1320	Butcher	Unknown	0.72	227	0.69
Pyramimonas parkeae	Prasinophyceae	CCMP 724	Norris and Pearson	Coastal	0.69	587	0.66
Nannochloris atomus	Chlorophyceae	CCMP 509	Butcher	Coastal	0.69	14	0.63
Pycnococcus provasoli	Prasinophyceae	CCMP 1203	Guillard	Coastal	0.64	10	0.50
Tetraselmis sp.	Prasinophyceae	CCMP 1639	Lewin	Estuarine	0.27	300	0.68
Gymnodinium chlorophorum	Dinophyceae	DIN 3	Elbrächter & Schnepf	Coastal	0.20	3410	0.72
Prorocentrum mimimum	Dinophyceae	CCMP 1329	(Pavillard) Schiller	Coastal	0.52	833	0.56
Amphidinium carterae	Dinophyceae	CCMP 1314	Hulburt	Estuarine	0.52	514	0.53
Thoracosphaera heimii	Dinophyceae	CCMP 1069	(Lohm.) Kamptner	Oceanic	0.33	1353	0.57
Emiliania huxleyi	Prymesiophyceae	ASM 1	Probert	Oceanic	0.64	142	0.58
Gephyrocapsa oceanica	Prymesiophyceae	JS1A	Probert	Oceanic	0.79	142	0.64
Ditylum brightwellii	Bacillariophyceae	CCMP 358	(West) Grunow	Oceanic	0.43	6995	0.61
Thalassiosira weissflogii	Bacillariophyceae	CCMP 1336	(Gru.) Fryxell et Hasle	Coastal	0.98	930	0.56
Nitzschia brevirostris	Bacillariophyceae	CCMP 551	Hustedt	Estuarine	0.67	119	0.51
Thalassiosira eccentrica	Bacillariophyceae	CCMP 1800	(Ehr) Cleve	Estuarine	0.27	6627	0.62

[a]CCMP clones were purchased from the Culture Collection of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA. DIN 3 and coccolithophores were provided from Dr. Ian Probert (Universite de Caen Basse Normandie, France). The provenance information of all CCMP clones were obtained from the CCMP website http://ccmp.bigelow.org; the information of ASM1 and JS1A were given by Robert Guillard (Bigelow Laboratory for Ocean Sciences).

metal grade hydrochloric acid (1.2 N, Fisher, Fairlawn, NJ, USA) at 70° C overnight and were rinsed with Milli-Q H_2O (Millipore, Billerica, MA, USA) thoroughly.

Preparation of the culture medium. Cells were cultured in the medium Aquil prepared and sterilized according to Price et al. (1988/1989). Synthetic ocean water was enriched with sterile and metal-free 150 μM $NaNO_3$, 10 μM Na_2HPO_4, and 40 μM Na_2SiO_3, plus 0.1 μM vitamin B_{12}, 0.1 μM biotin, 20 μM thiamin, and 100 nM Na_2MoO_4. In the presence of 100 μM EDTA, total trace metal concentrations were as follows: Mn_T, 120 nM; Zn_T, 80 nM; Cu_T, 20 nM; Co_T, 50 nM; and Cd_T, 15 nM. These concentrations were calculated to yield unchelated concentrations of Mn′, 10 nM; Zn′, 20 pM; Cu′, 0.2 pM; Co′, 20 pM; and Cd′, 20 pM (Westall et al. 1986, Price et al. 1988/1989). These choices of unchelated (total inorganic) metal concentrations are explained in the Results and Discussion, which also describes an experiment to determine a suitable Fe′ for the culture medium. Because the constants for metal complexation by EDTA and major anions in seawater are not all precisely known, there is uncertainty regarding the exact unchelated concentrations in the medium (in particular, Cd′ may be higher than calculated). Comparison with other data sets requires careful matching of total metal and ligand concentrations. The unchelated Fe concentration, which depends on the light intensity because of the photoreduction of the FeEDTA complex, was calculated as $Fe' = 2.4 \times 10^{-3} \times Fe_T$ under the light conditions of the experiments of Table 2 (250 μmol photons $\cdot m^{-2} \cdot s^{-1}$) up to a saturating value of approximately 1000 pM (Hudson and Morel 1990, Sunda and Huntsman 1995a). In the dark, the Fe′ was calculated as $Fe' = 1.2 \times 10^{-3} \times Fe_T$ (Sunda and Huntsman 1995a). Thus, under a 12:12-h light:dark cycle, the average Fe′ was calculated as $Fe' = 1.8 \times 10^{-3} \times Fe_T$.

Culturing and sampling. Phytoplankton species investigated in this study included six "green algae" (*Dunaliella tertiolecta, Pyramimonas parkeae, Nannochloris atomus, Pycnococcus provasoli, Tetraselmis* sp., and *Gymnodinium chlorophorum*), three "red" dinoflagellates (*Prorocentrum minimum*, *Amphidinium carterae*, *Thoracosphaera heimii*), two coccolithophores (*Emiliania huxleyi*, *Gephyrocapsa oceanica*), and four diatoms (*Ditylum brightwellii, Thalassiosira weissflogii, Nitzschia brevirostris, Thalassiosira eccentrica*) (Table 1). The algae were grown at 19 ± 1° C using a 12:12-h light:dark cycle with a cool white 250 μmol photons $\cdot m^{-2} \cdot s^{-1}$ light intensity. Replicate culture samples (n = 3–6) were maintained in exponential growth through a minimum of six generations before harvesting. Cell density and size were determined daily with a Coulter (Beckman Coulter, Hialeah, FL, USA) particle counter to calculate growth rates and cell volumes. The maximum quantum yield of photochemistry in PSII (F_v/F_m) was measured using a Fast Repetition Rate fluorometer (Chelsea Instruments, Surrey, U.K.) (Kolber et al. 1998). We used F_v/F_m as a proxy for the physiological status of the cells during the experiments (Falkowski and Raven 1997).

Harvesting and digestion of samples. Cells were harvested around noon (10 a.m. to 3 p.m.) with a six-channel plastic filtration manifold consisting of polypropylene Swinnex 25-mm in-line filter holders with Teflon o-rings (Millipore, Billerca, MA, USA) and 60-mL polypropylene syringes as funnels. Nuclepore (Whatman, Maidstone, UK) or Poretics (GE Osmonics, Minnetonka, MN, USA) polycarbonate filters with either 0.8- or 5-μm pore size were used depending on cell size. Samples (100–150 mL) were filtered under low vacuum (50–150 mm Hg, depending on the rigidity of the cells) and rinsed three times with 20 mL of 0.45 M NaCl solution or synthetic ocean water previously cleaned through a Chelex-100 column (Chelex, Rochester, NY, USA). Filtered samples were then digested in 10-mL Teflon (Teflon, Rochester, NY, USA) centrifuge vials (Nalgene) or 20-mL Teflon bottles with 90% HNO_3 (Fisher, trace metal grade or Optima grade) and 10% hydrofloric acid (Optima grade) at 120° C for 4 h (Cullen and Sherrell 1999).

HR-ICPMS. All elements but carbon and nitrogen were determined with a sector field HR-ICPMS (*Element 2*, ThermoFinnigan, San Jose, CA, USA) fitted with a self-aspiring microflow Teflon nebulizer (PFA-100, Elemental Scientific Inc, Omaha, NB, USA) and a quartz Scott-type double-pass spray chamber. Except for K, which was analyzed at high resolution ($m/\Delta m \cong 13{,}000$), the analysis was conducted at medium resolution ($m/\Delta m \cong 5000$). Sensitivity and stability of the machine were adjusted to optimize conditions before sample analysis. The sensitivity was around $1–2 \times 10^6$ counts per second for 10 ppb In under medium resolution. For the elements with more than one stable isotope, two isotopes were measured to confirm low interference: Mg 24/26, Ca 42/44, S 32/34, Fe 56/57, Cu 63/65, Zn 64/66, Sr 86/88, Mo 95/98, and Cd 110/111. The concentration differences between the two isotopes were less than 5% for all these elements. For the two coccolithophores and *T. heimii* samples, which had extremely high Ca contents, the matrix effect of Ca on the analysis of other trace metals was determined and corrected (approximately 5%–20% signal suppression).

Calibration, accuracy, and precision of the HR-ICPMS method. The HR-ICPMS instrument was calibrated using multi-element standard solutions prepared by mixing and diluting single element standards (High-Purity Standards, Charleston, SC, USA). Analysis of plankton reference material CRM414 (BCR, Commission of the European Communities) gave values that were within 10% the certified values (Mn 93%, Co 100%, Cu 102%, Zn 100%, Cd 100%, Fe not certified in CRM 414). The detection limits for the trace metals of interest—determined as three times the SD of filter blanks (n = 6) digested and analyzed as above—were as follows: Mn 0.09 nM, Fe 3 nM, Co 0.06 nM, Cu 1 nM, Zn 1 nM, Mo 0.2 nM, and Cd 0.03 nM. More than 95% of the concentrations measured in this study were at least one order of magnitude higher than the detection limits.

To verify the accuracy of the method from the growth of the cultures to the analysis of the elements, we compared Fe quotas obtained by HR-ICPMS with those obtained by liquid scintillation counting (LS counter 1801, Beckman, Palo Alto, CA, USA) of ^{55}Fe used as a tracer following the method of Granger and Price (1999). The cultures were grown under continuous light at 120 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (so that the formula for unchelated Fe is $Fe' = 1.8 \times 10^{-3}$ Fe_T up to the point of precipitation). Both total cellular Fe (without Ti-citrate-EDTA rinse) and intracellular Fe (with Ti-citrate-EDTA rinse) were measured in triplicate *T. weissflogii* cultures grown at five Fe′ concentrations (0.05, 0.14, 0.5, 1.4, and 14 nM) and harvested in late exponential phase by the two independent methods. As seen in Figure 1, the precision of the HR-ICPMS data (relative SE, 11%) is somewhat better than the precision of the radiotracer data (relative SE, 21%). The results for the two techniques follow the 1:1 line, thus validating the accuracy of the two methods.

Carbon and nitrogen analysis. Triplicate samples for carbon and nitrogen analysis were obtained by filtering 25- to 50-mL culture samples onto precombusted 13-mm GF/F filters or Gelman AE GF filters (ThermoFinnigan, San Jose, CA, USA). These were analyzed using a Carlo Erba elemental analyzer (Whatman, Maidstone, UK). Additional C and N analysis were performed for species whose cell walls contain calcium carbonate (*T. heimii*, *E. huxleyi*, and *G. oceanica*) or carbonhydrate (*P. parkeae*). The filters were fumed for 24 h with concentrated HCl to remove extracellular calcium carbonate or carbohydrate before C and N analysis.

TABLE 2. Elemental quotas and cellular concentrations of the marine phytoplankton.

	mol/mol P±1 S.E. ***mmol/L (bold and italics)***														
Species	C[a]	N	P	S	K	Mg	Ca	Sr[a]	Fe	Mn	Zn	Cu	Co	Cd	Mo
Dunaliella tertiolecta	222±32 ***11000***	38±5.6 ***1900***	1 ***49***	0.28±0.03 ***14***	0.36±0.08 ***18***	0.37±0.03 ***18***	0.019±0.002 ***0.94***	0.081±0.02 ***4.0***	11.3±0.48 ***560***	1.9±0.07 ***93***	1.49±0.08 ***74***	0.67±0.034 ***33***	0.010±0.0004 ***0.51***	0.10±0.006 ***5.1***	0.011±0.003 ***0.52***
Pyramimonas parkeae	475/(209±43) ***15454/(6800)***	17.6±4.6 ***570***	1 ***32***	1.44±0.28 ***47***	0.85±0.12 ***27***	N.A. ***N.A.***	1.69±0.30 ***55***	12±0.81 ***390***	15.4±2.18 ***500***	7.7±1.03 ***250***	1.47±0.12 ***48***	0.62±0.039 ***20***	0.24±0.028 ***7.6***	0.65±0.057 ***21***	0.035±0.003 ***1.1***
Nannochloris atomus	173±8 ***14000***	25±1.4 ***2000***	1 ***81***	0.36±0.02 ***29***	0.97±0.02 ***78***	0.24±0.01 ***19***	0.028±0.002 ***2.3***	0.046±0.01 ***3.7***	13.6±0.42 ***1100***	1.2±0.05 ***93***	1.68±0.14 ***140***	0.23±0.006 ***19***	0.081±0.002 ***6.5***	0.007±0.0008 ***0.60***	0.009±0.001 ***0.76***
Pycnococcus provasoli	191±34 ***14000***	27±5.0 ***1900***	1 ***72***	1.07±0.03 ***77***	1.23±0.09 ***89***	0.27±0.01 ***19***	0.055±0.006 ***3.9***	0.11±0.01 ***7.9***	12.6±0.85 ***910***	2.1±0.04 ***150***	0.92±0.062 ***66***	0.53±0.039 ***38***	0.090±0.002 ***6.5***	0.029±0.0016 ***2.1***	0.017±0.001 ***1.2***
Tetraselmis sp.	199±43 ***N.A.***	26±5.3 ***N.A.***	1 ***N.A.***	1.33±0.01 ***N.A.***	1.47±0.04 ***N.A.***	0.23±0.01 ***N.A.***	0.86±0.019 ***N.A.***	25±1.01 ***N.A.***	3.9±0.42 ***N.A.***	4.0±0.25 ***N.A.***	0.44±0.075 ***N.A.***	0.51±0.009 ***N.A.***	0.112±0.018 ***N.A.***	0.15±0.007 ***N.A.***	0.028±0.006 ***N.A.***
Gymnodinium chlorophorum	137±40 ***18000***	16.6±3.4 ***2000***	1 ***120***	N.A. ***N.A.***	N.A. ***N.A.***	N.A. ***N.A.***	N.A. ***N.A.***	N.A. ***N.A.***	14.4±0.90 ***1700***	1.9±0.08 ***220***	0.87±0.070 ***110***	1.36±0.18 ***160***	0.24±0.02 ***29***	0.097±0.010 ***12***	0.11±0.02 ***13***
Prorocentrum mimimum	135±21 ***22000***	11±1.9 ***1800***	1 ***16***	2.18±0.05 ***350***	1.29±0.01 ***210***	0.99±0.16 ***160***	0.38±0.070 ***61***	2.9±0.5 ***470***	6.8±1.72 ***1100***	6.1±0.18 ***980***	0.89±0.063 ***140***	0.28±0.012 ***440***	0.46±0.0053 ***73***	0.12±0.005 ***19***	0.025±0.01 ***4.0***
Amphidinium carterae	126±24 ***1200***	18±3.8 ***160***	1 ***9.1***	1.34±0.09 ***12***	0.15±0.05 ***1.4***	0.57±0.04 ***5.3***	0.27±0.027 ***2.5***	1.2±0.08 ***11***	13.3±0.37 ***120***	5.1±0.16 ***47***	1.26±0.310 ***12***	0.54±0.059 ***5.0***	0.35±0.010 ***3.2***	0.73±0.037 ***6.7***	0.11±0.010 ***0.98***
Thoracosphaera heimii[a]	112/(81±20) ***5100/(5100)***	6.4±1.4 ***400***	1 ***63***	1.31±0.04 ***82***	1.01±0.01 ***63***	0.48±0.01 ***30***	45±1.1 ***2800***	79±1.7 ***5000***	1.8±0.2 ***110***	1.3±0.09 ***79***	0.11±0.07 ***7.0***	0.06±0.014 ***3.5***	0.095±0.013 ***5.9***	0.11±0.0057 ***6.8***	0.039±0.026 ***2.5***
Emiliania huxleyi[a]	237/(76±23) ***31000/(10000)***	8.8±2.9 ***1200***	1 ***130***	0.77±0.02 ***100***	0.84±0.02 ***110***	0.13±0.004 ***18***	142±7.0 ***19000***	336±10 ***44000***	3.5±0.07 ***460***	7.1±0.36 ***940***	0.38±0.002 ***50***	0.07±0.013 ***8.9***	0.29±0.020 ***39***	0.36±0.0097 ***48***	0.022±0.0003 ***2.9***
Gephyrocapsa oceanica[a]	224/(64±12) ***31150/(8900)***	7.3±1.3 ***1000***	1 ***140***	1.00±0.07 ***140***	0.95±0.02 ***130***	0.13±0.01 ***18***	127±3.1 ***18000***	278±3.3 ***39000***	4.0±0.87 ***560***	7.1±0.13 ***990***	0.41±0.088 ***57***	0.11±0.007 ***16***	0.360±0.010 ***50***	0.31±0.011 ***43***	0.023±0.005 ***3.2***
Ditylum brightwellii	45±18 ***5700***	5.4±1.0 ***690***	1 ***130***	2.82±0.13 ***360***	3.31±0.11 ***420***	N.A. ***N.A.***	1.05±0.11 ***130***	6.8±0.8 ***870***	0.3±(NA) ***43***	1.9±0.08 ***250***	0.07±0.023 ***9.2***	0.06±0.004 ***8.0***	0.17±0.005 ***22***	0.18±0.008 ***23***	0.014±0.002 ***1.8***
Thalassiosira weissflogii	86±7 ***12000***	13.6±1.1 ***1800***	1 ***140***	1.34±0.18 ***180***	5.30±0.26 ***720***	N.A. ***N.A.***	0.40±0.11 ***54***	2.2±0.74 ***300***	1.7±0.18 ***230***	5.3±0.08 ***720***	0.75±0.027 ***100***	0.17±0.005 ***23***	0.108±0.0014 ***15***	0.066±0.0008 ***8.9***	0.020±0.002 ***2.7***
Nitzschia brevirostris	42±11 ***11000***	6.8±2.1 ***1700***	1 ***250***	1.15±0.12 ***290***	2.43±0.18 ***610***	0.60±0.1 ***150***	0.27±0.062 ***67***	1.3±0.48 ***330***	3.1±2.0 ***790***	2.3±0.04 ***590***	0.27±0.041 ***69***	0.18±0.007 ***46***	0.056±0.0014 ***14***	0.019±0.0028 ***4.8***	0.014±0.002 ***3.6***
Thalassiosira eccentrica	75±10 ***18000***	7.8±1.0 ***1900***	1 ***240***	1.94±0.12 ***470***	3.25±0.22 ***790***	2.14±0.32 ***520***	0.65±0.035 ***160***	3.9±0.45 ***950***	6.7±0.18 ***1600***	2.1±0.06 ***500***	1.00±0.41 ***240***	0.28±0.069 ***68***	0.240±0.039 ***59***	0.15±0.006 ***36***	0.020±0.002 ***4.8***
Average	147(124)a ***11000***	16 ***1400***	1 ***120***	1.3 ***170***	1.7 ***250***	0.56 ***100***	23a ***3100***	54a ***7000***	7.5 ***700***	3.8 ***420***	0.80 ***80***	0.38 ***35***	0.19 ***24***	0.21 ***17***	0.033 ***3.1***
Relative SE (n = 11–16)	13% ***13%***	16% ***13%***	 ***17%***	14% ***26%***	23% ***31%***	31% ***53%***	57% ***61%***	55% ***61%***	18% ***20%***	16% ***23%***	17% ***21%***	24% ***31%***	17% ***26%***	28% ***25%***	25% ***28%***

For each element and species, the upper number is the elemental quota that is normalized to P; the lower italic number is the cellular concentration that is normalized to cellular volume. The units of quotas and concentrations for C, N, P, S, K, Mg, and Ca are mol/mol and mmol/L, respectively. The units for all other trace metals (Sr, Fe, Mn, Zn, Cu, Co, Cd, and Mo) are mmol/mol and μmol/L, respectively.

[a]The carbon data with brackets were obtained from samples treated with HCl. The average Ca and Sr data reported in the table are the values without HCl treatment. Without including the three high Ca/Sr species (*T. heimii*, *E. huxleyi*, and *G. oceanica*), the average quotas for Ca and Sr are 0.52 mol/mol and 5.0 mmol/mol P, respectively. N.A., not available.

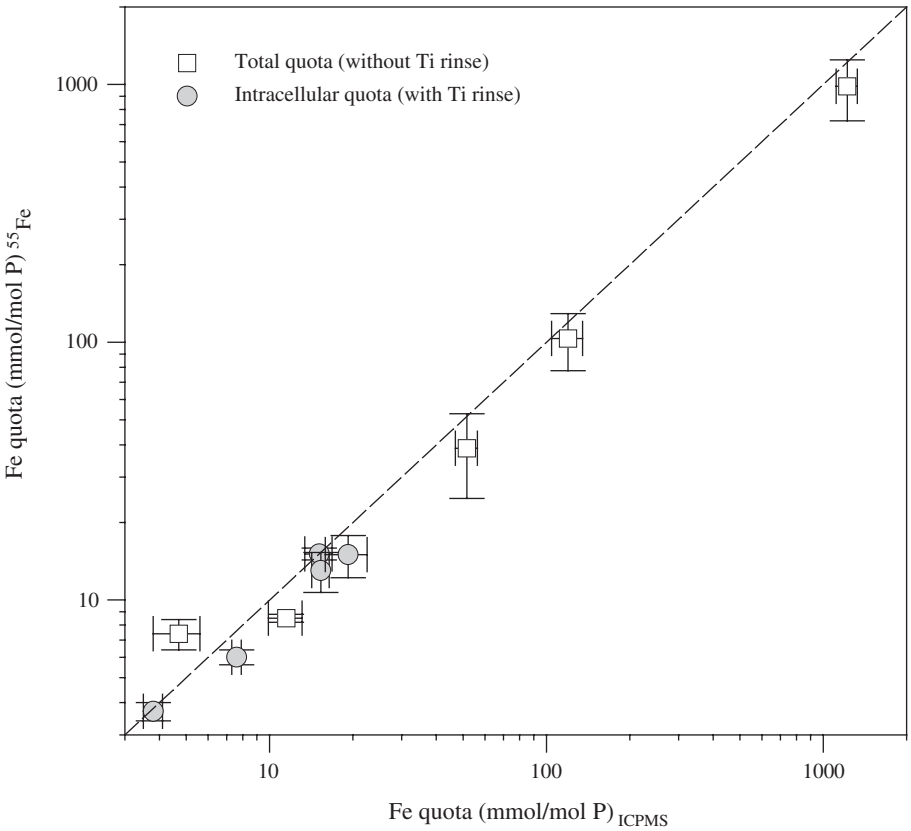

FIG. 1. Comparison of Fe quotas measured by two independent methods: HR-ICPMS and ^{55}Fe tracer. Triplicate *Thalassiosira weissflogii* cultures were grown at five different Fe′ concentrations (0.05, 0.14, 0.5, 1.4, and 14 nM) with either natural isotopes or ^{55}Fe. The Fe:P ratios for the HR-ICPMS method were directly obtained by HR-ICPMS. The Fe:P ratios of the ^{55}Fe tracer experiment were determined by multiplying the Fe:C ratio (determined from liquid scintillation counter and carbon hydrogen nitrogen elemental analyzer) by C/P = 106. These experiment were carried out under 24-h continuous light (120 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}).

RESULTS AND DISCUSSION

Choice of medium composition. To obtain systematic and meaningful data, we chose to use the same culture medium for all phytoplankton species and to buffer the unchelated trace metal concentration at relatively low values—generally near what are thought to be the unchelated concentrations in surface seawater—but not so low as to severely limit growth rates. Thus, as in the medium Aquil (Price et al. 1988/1989), the unchelated concentrations of Zn and Co (Zn′ and Co′ were maintained at 20 pM and that of Cu (Cu′) at 0.4 pM. We also added Cd to maintain at Cd′ = 20 pM. In previous studies of the effects of trace elements on a few species of phytoplankton, these concentrations have been shown to allow maximum growth rates (Brand et al. 1983, 1986, Brand 1991, Sunda and Huntsman 1995b). The low Cu′ concentration was chosen to avoid inhibition of uptake of other metals such as Mn (Sunda and Huntsman 1983). The typical unchelated concentrations reported for these metals in surface waters of the open ocean are in the range of 1–20 pM for Cd′ and Zn′ (Bruland 1989, 1992), 0.4–5 pM for Cu′ (Moffett 1995), and 0.1–100 fM for Co′ (Saito and Moffett 2001). To avoid limiting the growth of coastal species, Mn′ was set at 10 nM, a value that is typical of coastal waters and about 10 times higher than in the open ocean (Landing and Bruland 1980).

The most critical and difficult choice was that of an appropriate Fe concentration. On the one hand, low unchelated Fe concentrations buffered with EDTA usually lead to less than maximum growth rates even when they are higher than is estimated for open ocean surface water—approximately 0.05–1 pM (Rue and Bruland 1995). [This is presumably a reflection of the fact that some organically bound Fe in seawater is available to phytoplankton (Maldonado and Price 1999, Hutchins et al. 1999).] On the other hand, high Fe concentrations unavoidably result in precipitation of hydrous ferric oxide, FeO_x, onto cell surfaces (Morel and Hering 1993). Because FeO_x adsorbs other elements, this precipitation can lead to an overestimation of quotas of several trace metals in addition to Fe. Although it is possible to wash cells with a titanium-citrate-EDTA solution to remove the extracellular Fe before measuring the cellular metals (Hudson and Morel 1989), the wash solution contains high concentrations of trace metals like Ti, Cu, and Zn, which is problematic for multielemental analysis of phytoplankton. Other

washing procedures can be used, but all run the risk of not being sufficiently efficient, lysing the most fragile cells and contaminating the sample.

We thus measured the total and intracellular iron content (mol Fe per cell) in *T. weissflogii* growing over a range of Fe concentrations. The intracellular iron content (i.e. the cellular content measured after titanium-citrate-EDTA wash) increased with Fe′ between 0.1 and 1.0 nM and plateaued at approximately 15 mmol Fe/mol P as the total Fe concentration increased in the medium above the point of saturation of FeO_x (Fig. 2B). In contrast, the measured total cellular content kept increasing up to 1000 mmol/mol P, indicating that most of the measured cellular Fe was indeed extracellular and precipitated on the cell surface. To obtain good growth rates and minimize the problem of extracellular precipitation, we chose to use for our culture medium a total Fe concentration of 82 nM, corresponding to an unchelated Fe concentration, Fe′ = 0.14 nM (0.2 nM in the following experiments, which were performed at a higher light level). At this concentration, *T. weissflogii* grew at 85% of its maximum rate (Fig. 2A) and the extracellular Fe amounted to no more than 30% of the total Fe quota measured (Fig. 2C). [Note that about 5% of that 30% is not precipitated but bound to surface ligands and eventually taken up by the cells (Hudson and Morel 1990).] This choice of Fe′ thus allows one to obtain

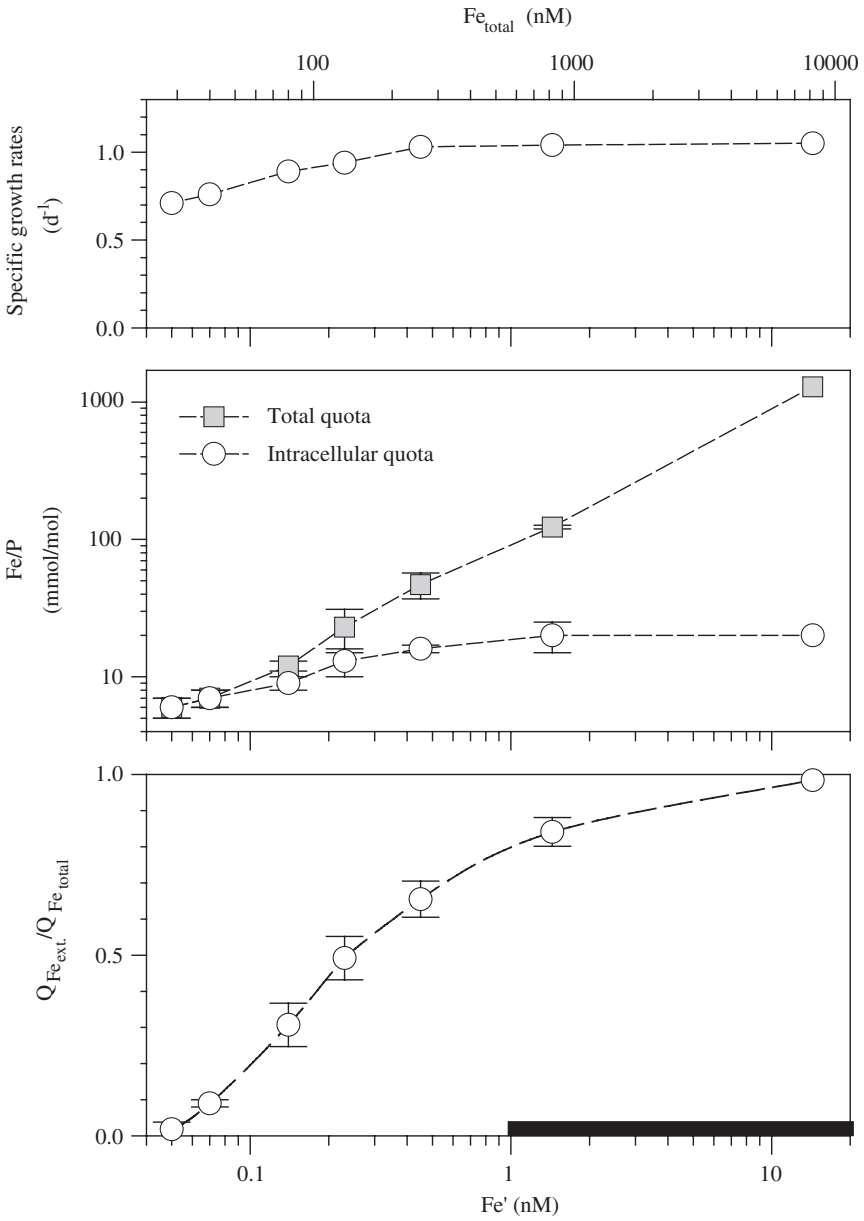

FIG. 2. Variations of growth rates and intracellular and total Fe quotas in *Thalassiosira weissflogii* grown at different Fe′. (C) Ratios of extracellular Fe quotas (on cell surface) to total Fe quotas. The black bar indicates the range of Fe′ over which hydrous ferric oxide is saturated. These experiment were carried out under 24-h continuous light (120 μmol photons · $m^{-2} \cdot s^{-1}$).

reasonably accurate values of cellular concentrations with no treatment of the cells other than a chelated salt water wash before digestion and measurement.

As can be seen in Table 1, most of the species we tested grew well under the conditions chosen for this study. Even the five species that grew relatively slowly (<0.5 day^{-1}) were photosynthesizing efficiently as evidenced by their high maximum quantum yields (F_v/F_m).

Elemental composition of phytoplankton. The elemental compositions measured for 15 species of marine phytoplankton are presented in Table 2 as mol ratios normalized to P (mol of element per mol P, referred to as "quotas") and, in italics, as molar concentrations (mol per liter of cell volume, referred to as "cellular concentrations"). Phosphorus normalized quotas are most often used by oceanographers (following Redfield 1934, 1958) and provide an easy comparison among organisms and with natural plankton samples independent of cell volumes. Significant interspecies variability of the quotas is observed for all elements with the variability ($n = 15$) ranging from 55%–57% (relative SE) for Sr and Ca to 13%–14% (relative SE) for C and S (see box and whisker graphs in Fig. 3). In addition, there appears to be systematic differences in the composition of the various phyla (Fig. 4).

Major cations: K, Mg, Ca, and Sr. The cellular quota of Na could not be measured in this study because we rinsed the cells with either an NaCl solution or synthetic ocean water before analysis. The cellular quotas of K and Mg in marine phytoplankton, which range over more than one order of magnitude, are surprisingly variable in view of the constant concentrations of these metals in seawater. The cellular concentrations of K are higher than in seawater, whereas those of Mg are generally lower. Diatoms have remarkably high concentrations of both K and Mg, their cellular Mg concentrations being in fact higher than in seawater.

Not surprisingly, a very high quota is observed for Ca in calcifying species. The calcium carbonate in the cell walls of these organisms augments markedly both their Ca and their C quotas. For example, the "hard parts" of coccolithophores may contain as much C as their "soft parts." To get a better elemental stoichiometry for organic biomass, we reanalyzed the C content of the calcifying species after fuming the samples with concentrated HCl (parenthetical values in Table 2). The resulting average C quotas and the average Ca quotas calculated for noncalcifying species (without including *T. heimii*, *E. huxleyi*, and *G. oceanica*) provide an estimate of the stoichiometric composition of organic biomass for the average phytoplankton (parenthetical numbers in the "average" of Table 2).

The Sr quotas of the coccolith-forming species are much higher than those of other species. Indeed, the coccoliths have been shown to contain relatively high Sr concentrations. The Sr:Ca ratio we observed in *E. huxleyi* (2.4 mmol/mol) is similar to that previously reported for the same organism (2.5–3.2 mmol/mol; Stoll et al. 2002). The average Sr quota of 5.0 mmol/mol P in noncalcifying species presumably provides a good approximation of the Sr content of the organic biomass. [In the following discussion, we assume implicitly that the other elements of interest, contrary to Sr, are present chiefly in the soft parts of phytoplankton. This assumption has been shown to be correct for a few elements in a few species of diatoms and coccolithophores; e.g. Fe and Zn in frustules (Ellwood and Hunter 2000) and Cd in coccoliths (unpublished data).]

Major nutrients: C, N, P, and S. The quotas for the major nutrients, C, N, and S, in the organic biomass are quite variable, with differences by factors of 5, 7, and 10, respectively, among individual species. Nonetheless, the average quotas of the organic biomass (C:N:P:S = 124:16:1:1.3) are close to those reported by Redfield et al. (1963), based on the data of Fleming (1940).

The good match between our average N quota and the "canonical" Redfield quota of 16 may seem surprising given that a recent compilation of the published data on the composition of marine phytoplankton in culture showed that the average measured N:P ratios are markedly lower (Geider and La Roche 2002). A perusal of the references cited in Geider and La Roche (2002) reveals that most of the species in these previous studies were diatoms and dinoflagellates, which we also found to have relatively low N:P ratios (Fig. 4). The high average quota we obtained may thus simply represent a difference in the collection of species we studied compared with previous investigations, in particular the inclusion of five species of green algae (*Chlorophyceae* and *Prasinophyceae*) that have high N:P ratios. Thus, the good match between our average N:P ratio and the canonical Redfield value, which comes from measurements of nutrients dissolved in seawater and in field samples of suspended material, seems to be fortunate.

Under our culture conditions, the C:P ratios of green algae, like their N:P ratios, are much higher than those of diatoms and of the soft parts of coccolithophores. But as noted by Geider and La Roche (2002), the C:N ratios in the organic fraction of all species are relatively constant in the range 6 to 9. Some dinoflagellates with carbohydrates in their cell walls have somewhat higher C:N ratios.

Our average S quota of 1.3 is significantly higher than that estimated from the limited literature (0.35 ± 0.16; Payne and Price 1999) and somewhat lower than reported by Redfield et al. (1963, S/P = 1.7) for field samples. The S content of the cells reflects chiefly the concentration of cysteine, glutathione, and derived compounds in addition to dimethylsulfoniopropionate. Because these S-containing compounds are often involved in metal binding, their concentration may be influenced by the trace metal chemistry of the medium. It may thus be significant that high S quotas were observed in both our cultures and field

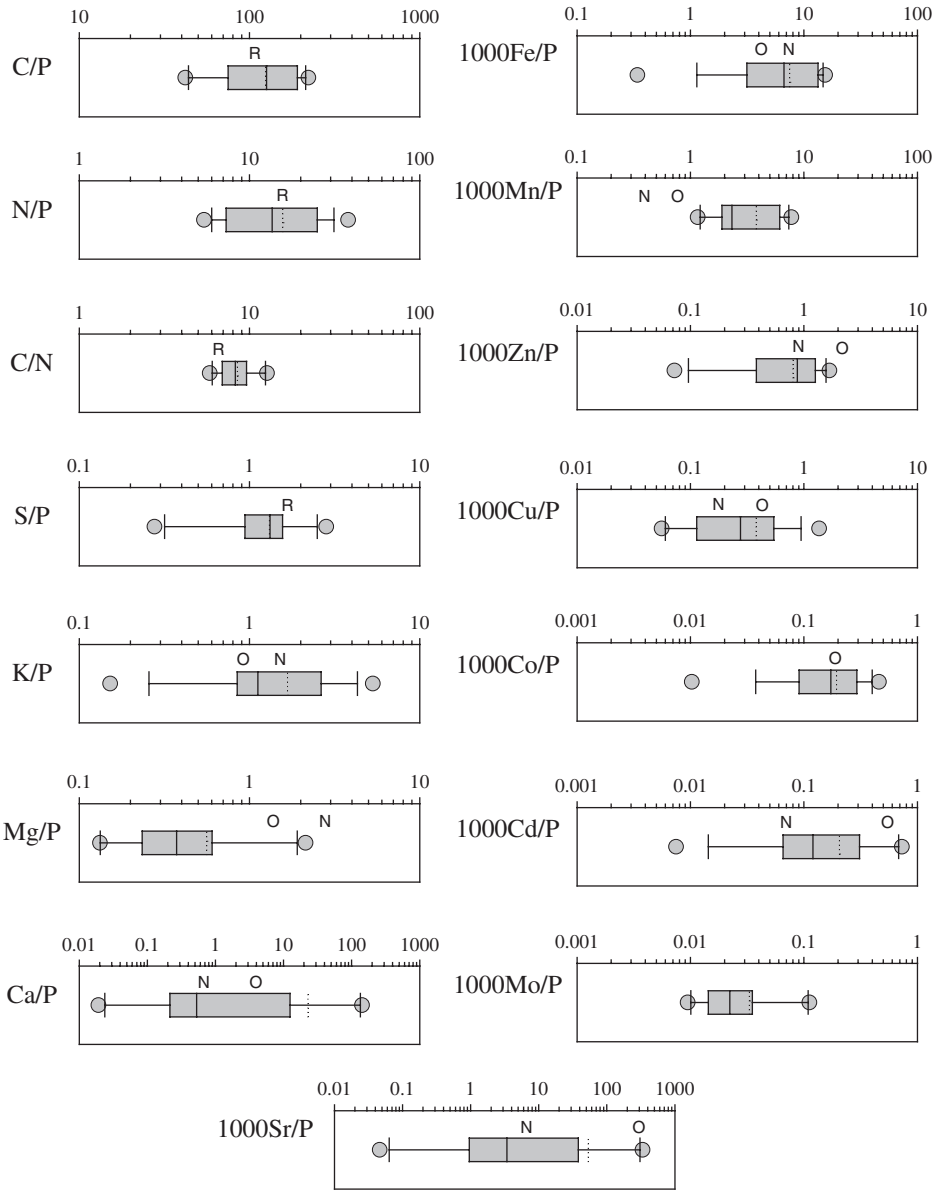

FIG. 3. Box and whisker graphs of individual elemental quotas for all phytoplankton analyzed in this study. The value of the Redfield ratio and the elemental quotas in planktonic or suspended particular materials in the field studies listed in Table 4 are also labeled on the graphs with the letters R (Redfield ratio), N (quotas from the neritic study), and O (average quotas from the three oceanic studies). Details of these studies are explained in Table 4. The units are mol/mol P for C, N, S, K, Mg, and Ca and mmol/mol P for Sr, Fe, Mn, Zn, Cu, Co, Cd, and Mo. The two ends of each bar represent 10th and 90th percentiles for all data points. The two ends of each box represent the 25th and 75th percentiles of all data points. The solid lines in each box represent the means for the data in each box (25th to 75th percentiles) but not the mean of all data points. The dashed lines in each box represent the means of all data points. The data lying outside the 10th and 90th percentiles are plotted as circles.

samples but not in previous studies where the trace metal concentrations were presumably much higher.

Given the variability of the individual values, it is perhaps surprising that our average quotas for major nutrients (particularly the N:P ratio) are so close to the Redfield values. This is partly the result of averaging a sufficiently large number of data as remarked by Redfield in 1934: his C:N ratios for plankton samples varied from 4.6 to 14.4 and his N:P ratios from 13.3 to 53.5 (at a time when the corresponding "Redfield ratios" were 7 and 20). Newer field data also show considerable deviations from Redfield, with C:N ratios, for example, reaching values of 14 (Sambrotto et al. 1993) but N:P ratios being somewhat less variable. The question of why the N:P ratio is approximately16 in seawater and how it is maintained at this particular average value is still clearly of interest to oceanographers (Falkowski 2000, Geider and La Roche 2002).

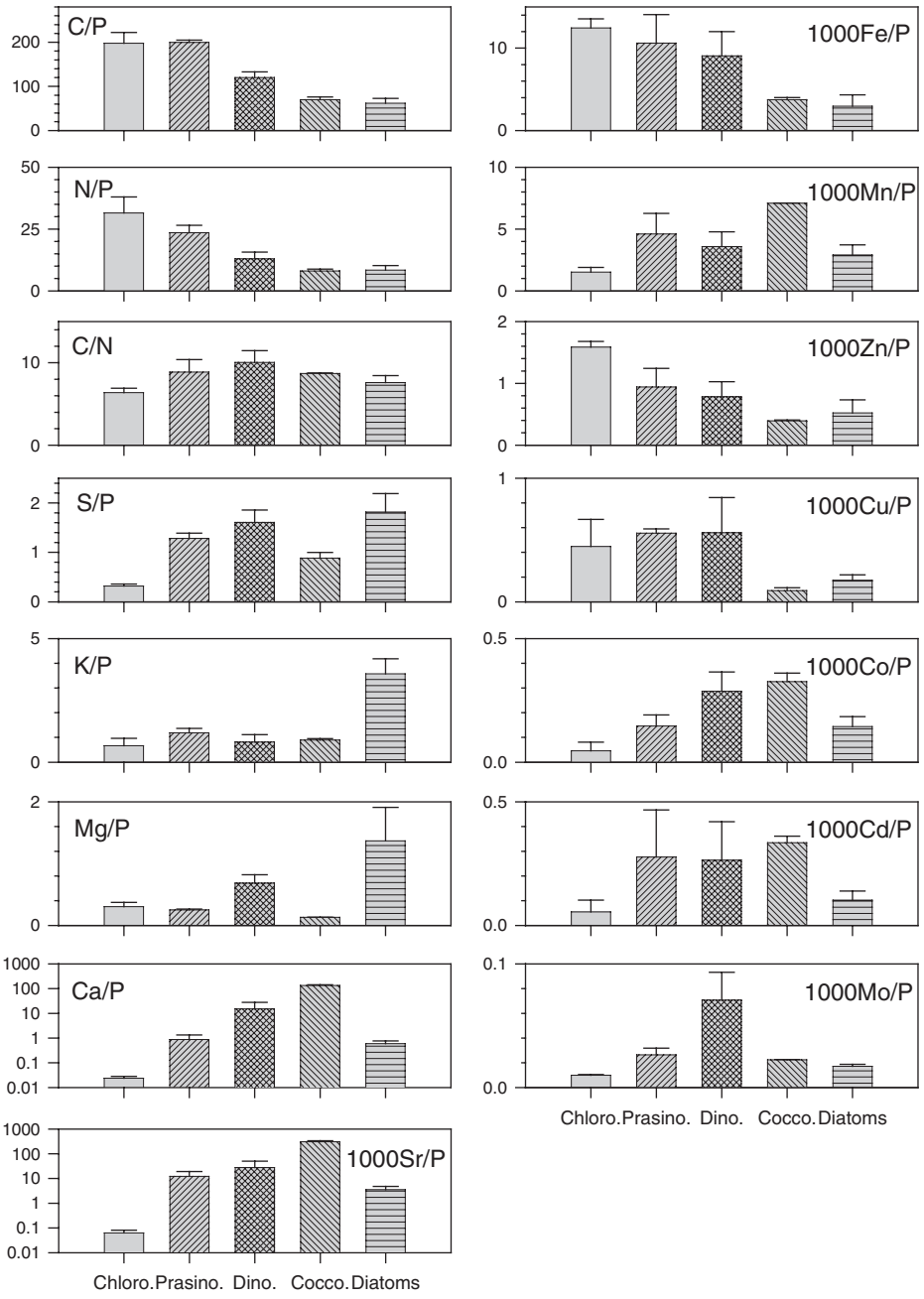

FIG. 4. Comparison of the average elemental quotas among different phyla. The bar graphs of C:P, C:N, and N:P are adapted from Quigg et al. (2003). Except for C:N, all elements are normalized to P (mol/mol or mmol/mol). For $n > 2$, the error bars represent 1 SE; for $n = 2$, the error bars represent 1 average deviation. Chlorophyceae, $n = 3$; Prasinophyceae, $n = 3$; Dinophyceae, $n = 4$; Prymesiophyceae, $n = 2$; Bacillariophyceae, $n = 4$.

Trace metals: Fe, Mn, Zn, Cu, Co, Cd, and Mo. As expected, the trace metal quotas exhibit greater variability among species than the major nutrients. Nonetheless, for each element, most of the quotas (10th to 90th percentile) are within a factor of 20 of each other, except for Cd quotas, which range over two orders of magnitude. In most species tested, the cellular concentrations follow the order $Fe > Mn > Zn > Cu > Co \approx Cd > Mo$. The average cellular concentrations of Fe, Cu, and Mo in phytoplankton (Table 2) are similar to those reported for *Escherichia coli* (Outten and O'Halloran 2001), but the concentration of Mn is almost 100 times higher and that of Zn 10 times lower. As discussed below, the high Mn concentrations in our samples may reflect in part the high unchelated Mn concentration in our growth

medium. In addition, a high cellular Mn concentration in phytoplankton compared with bacteria is expected in view of the high Mn requirements for the oxygen-evolving complex of PSII. The low cellular Zn concentrations presumably reflect the extremely low concentration of Zn in our medium and in seawater. The average quotas for Co and Cd, which are about one third of the average cellular Zn concentration, seem quite high in view of the few documented biochemical use of Co and Cd compared with Zn. Both these high Co and Cd quotas and the low Zn quotas may reflect in part a biochemical substitution of Co and Cd for Zn in marine phytoplankton (Price and Morel 1990, Morel et al. 1994, Sunda and Huntsman 1996).

The most systematic differences in trace element quotas among classes of phytoplankton are seen between the green algae and the diatoms and coccolithophores: The green algae, particularly the chlorophytes, have relatively high Fe, Zn, and Cu quotas and low Mn, Co, and Cd quotas compared with the diatoms and coccolithophores. These differences between the "green" and "red" superfamilies of phytoplankton have been discussed by Quigg et al. (2003) in the context of evolutionary histories. Also of interest are the higher Mn, Co, and Cd quotas in coccolithophores compared with diatoms. Because all our coccolithophore isolates are from open ocean waters and most of our diatom isolates are from coastal or estuarine waters, these differences could also reflect the known differences in metal requirements between oceanic and neritic species (Brand et al. 1983). The one diatom isolated from open ocean waters (*Ditylum brightwellii*, isolated from the Gulf of Mexico) has markedly lower Fe, Mn, and Cu quotas than the other diatoms and relatively high Co and Cd quotas. Low Fe and Mn requirements and quotas for oceanic isolates have been previously reported (Sunda and Huntsman 1997).

Because they have been obtained from cultures growing under identical conditions, our data provide useful information on the relative elemental composition of various phytoplankton species. But to what extent do these data reflect the biochemistry of the organisms rather than the composition of the growth medium or the culture conditions? Further, is the variability in elemental composition that is seen among species under one set of conditions greater or smaller than the variability within one species under a range of growth conditions? The answer to these questions is provided by laboratory studies in which the quota of a given trace metal in a given phytoplankton species is measured under a variety of conditions, most importantly over a range of unchelated metal concentrations. Such detailed studies have been performed by Sunda and Huntsman (1995b, 1998b, 2000), who provided extensive trace metal data for two of the species that are included in our study—the estuarine diatom *T. weissflogii* and the open ocean coccolithophore *E. huxleyi*—and very complete data for the coastal diatom *Thalassiosira pseudonana*. To compare our data with their results, we converted the -log free metal concentrations (pM) given by Sunda and Huntsman to unchelated concentrations (M′) (Table 3). Because the cellular P concentrations were not measured in the studies of Sunda and Huntsman, we also converted the reported cellular metal:C molar ratios to metal:P ratios by multiplying by 106.

As seen in Table 3, the cellular trace metal quotas in phytoplankton are generally well regulated over a certain range of bioavailable metal concentrations. For example, an increase by a factor of 100 in an unchelated metal concentration typically results in a much smaller increase in the corresponding cellular quota: a factor of only 8 for Fe in *E. huxleyi* and factors of 6 and 3, respectively, for Zn and Cd in *T. weissflogii*. The least regulated metals in Table 3 appear to be Co and Cd in *E. huxleyi* for which increases by factors of 30 in Co′ and 100 in Cd′ in the culture medium lead to increases in the corresponding quotas by factors of 6 and 20.

With the exception of Cd, our metal quotas for the same species fall within or quite near the regulated values reported by Sunda and Huntsman (or in the range reported for *T. pseudonana* if there is no range of values available for *T. weissflogii*). Our quotas are also generally close to those reported by (or extrapolated from) Sunda and Huntsman for the same unchelated metal concentrations, again with the exception of our Cd quotas, which are roughly a factor of 2 lower. It must be noted that an agreement within a factor of 2 is not unreasonable in such experiments for, in addition to analytical difficulties, a large number of culture parameters affect the measured cellular quotas, as now discussed.

In addition to the bioavailable metal concentration in the medium, the quota of a trace metal in phytoplankton may depend on the light regime, the concentrations of major nutrients, and the concentrations of other trace metals. Light affects chiefly the cellular concentration of Fe, which is an integral part of a host of electron carriers involved in photosynthesis. For example, a change in light intensity from 50 to 500 μmol photons $\cdot m^{-2} \cdot s^{-1}$ was observed to result in a decrease by a factor of 2 in the Fe quota of the dinoflagellate *Prorocentrum minimum* (Sunda and Huntsman 1997). The concentration and chemical form of nitrogen in the medium also influences Fe requirements and quotas in phytoplankton. For example, *T. weissflogii* cultures growing on nitrate were found to have a 60% higher Fe quota than cultures grown on ammonium (Maldonado and Price 1996).

The concentrations of trace metals that compete for cellular uptake or that can substitute, in particular, biochemical functions also affect metal quotas. For example, the cellular concentration of Mn is highly dependent on the external concentrations of Cu, Zn, and Cd that interfere with Mn uptake. But this effect have only been seen in culture at high concentrations of unchelated metals (Cu′, Cd′, or Zn′ > 5 nM). Because

TABLE 3. Comparison of measured trace metal quotas for *T. weissflogii*, *T. pseudonana*, and *E. huxleyi* with values reported by Sunda and Huntsman.

		Thalassiosira weissflogii (*T. w.*) *or* *Thalassiosira pseudonana* (*T. p.*)			*Emiliania huxleyi*	
		Sunda and Huntsman		This study	Sunda and	
Element	M′ and quota (mmol/mol P)	(*T. w.*)	(*T. p.*)	(*T. w.*)	Huntsman	This study
Fe	Fe′ (pM)	271–750	244–760	150	7–750	150
Q_{Fe}	2.3–3.3[b]	3.1–7.4[b]	1.7	0.6–4.8[b]	3.5	
Mn	Mn′ (nM)	N.A.	5–140	10	N.A.	10
Q_{Mn}		2.3–3.4[c,d]	5.3		7.1	
Zn	Zn′ (pM)	25–2500	8–245	20	0.3–100	20
Q_{Zn}	0.5–3.2[e]	0.36–0.86[e]	0.75	0.03–5[f]	0.38	
Cu	Cu′ (pM)	18	0.2–200	0.4	0.03–6	0.4
Q_{Cu}	0.05[g]	0.24–2.1[g]	0.17	0.10–0.5[g]	0.07	
Co	Co′ (pM)	N.A.	0.06	20	0.5–17	20
Q_{Co}		0.01[f]	0.11	0.07–0.4[f]		0.29
Cd	Cd′ (pM)	8–813	389	20	8–813	20
Q_{Cd}	0.19–0.60[h]	0.1–0.3[h]	0.07	0.9–19[h]	0.36	

Only data with $\mu > 80\%\ \mu_{max}$ were selected. The range of unchelated (M′) metal concentrations in the studies of Sunda and Huntsman was calculated from free metal concentrations (pM) given in their papers and the conversion factors[a] listed in the footnote.

[a]The values of Fe′ were reported in the Table 1 of Sunda and Huntsman (1995a). The conversion formulae between M′ and pM for other metals are $Mn' = 10^{(-pMn+0.15)}$, $Cu' = 10^{(-pCu+1.3)}$, $Zn' = 10^{(-pZn+0.39)}$, $Co' = 10^{(-pCo+0.23)}$, and $Cd' = 10^{(-pCd+0.92)}$. Assume C/P = 100.

[b]Data are from Table 1 in Sunda and Huntsman (1995a).

[c]Mn quota for *T. pseudonana* was obtained at pCu = 12.8 from Figure 3 in Sunda and Huntsman (1983).

[d]Data from Figures 1 and 2 in Sunda and Huntsman (1996). Mn quotas for *T. pseudonana* were obtained as pZn ranging from 9.99 to 8.99 and pCd ranging from 9.3 to 12.7.

[e]*T. weissflogii* data are from Figure 3 in Sunda and Huntsman (1992). *T. pseudonana* data are from Table 1 in Sunda and Huntsman (1995b).

[f]Zn quotas were obtained under pCo ranging from 11.03 to 12.49 in Table 1 of Sunda and Huntsman (1995b). Co quotas for *T. pseudonana* were obtained at pZn = 10.99. Co quotas for *E. huxleyi* were obtained at pZn = 12.

[g]Sunda and Huntsman (1995c)

[h]Cd quotas both for *T. weissflogii* and *E. huxleyi* were obtained at pZn = 11.38 (Zn′ = 10 pM) from the Table 1 in Sunda and Huntsman (2000). Cd quotas for *T. pseudonana* were obtained from Figure 4 of Sunda and Huntsman (1996) under pMn ranging from 7.8 to 9.3, pCo = 10.9, and pZn = 8.33 (Table 1).

of biochemical metal substitution, the cellular concentrations of Zn, Co, and Cd in phytoplankton are interdependent. For example, the Co quota of *E. huxleyi* decreased by a factor of 2 as Zn′ increased from 1 to 25 pM (Sunda and Huntsman 1995b) and the Cd quota of *T. weissflogii* decreased 5-fold when Zn′ increased from 10 to 100 pM (Sunda and Huntsman 2000). In the case of diatoms, Zn, Co, and Cd are known to substitute for each other in carbonic anhydrases that are involved in inorganic carbon acquisition (Morel et al. 1994, Lee et al. 1995, Yee and Morel 1996). The Co and Cd quotas in these organisms thus also depend on the P_{CO_2} in the growth medium. Under elevated P_{CO_2} (750 ppm), the Co quotas of diatoms typically decrease by a factor of 2 (unpublished data). As a result of such metal substitutions, we should expect relatively high cellular concentrations of Co and Cd in open ocean phytoplankton growing at very low Zn concentrations.

Overall, it appears that in most instances the trace metal quotas of individual phytoplankton are highly regulated. The concentrations of major nutrients and other trace elements in the medium or the light intensity rarely change the cellular quota of a given trace metal by more than a factor of 2 to 5. Even the bioavailable concentration of a trace metal usually has a relatively modest effect on its cellular quota that is regulated within a factor of 5 to 10 when the free metal concentration varies over a "regulated range" spanning up to two or three orders of magnitude. Table 2 can thus be taken as a first approximation of the "normal" elemental quotas of various species of phytoplankton. In particular, the differences that are seen among species, which span one or two orders of magnitude depending on the metal, should reflect the individual biochemical requirements for essential elements as well as the ability of the organisms to take up and store these elements.

Average composition and comparison with field data. The species included in Table 2 should not be considered representative of the planktonic biomass in the oceans. As noted by Redfield in 1934, "unfortunately no adequate means of obtaining a truly representative sample of the entire population is available." But the range of biochemical possibilities represented by the species included in our study may approximate the variability found in nature. This is particularly true of the photosynthetic machinery that, as implied by the analysis of Quigg et al. (2003), may be responsible for much of the trace metal

requirements of phytoplankton. This can be verified by comparing our data with the composition of suspended particulate matter in seawater reported by various authors (Table 4). We selected for comparison the data sets that had sufficient information (e.g. P concentrations along with trace element concentrations) and, within those data sets, the samples that had a low fraction of lithogenic material as witnessed by low Al concentrations. [Following Bruland et al. (1991), we chose a cutoff of Al < 100 μg/g dry weight, which corresponds to approximately 1 μmol Fe/g dry weight in crustal rock, equal to the lowest cellular Fe concentration in Table 2.]

The coastal data set of Martin and Knauer (1973) came from plankton tows, as did the open ocean samples of Martin et al. (1976) and Collier and Edmond (1984). The data of Kuss and Kremling (1999) came from samples collected via pumping and centrifugation. As can be seen in Table 4, these newer open ocean data are nonetheless remarkably consistent with the earlier values. (One should note that all of these studies must have undersampled the picoplankton and thus the cyanobacteria, no species of which is included in this study.)

For comparison with the laboratory data, we included in Figure 3, for each cation and trace metal, the coastal water quota (indicated by the letter N for "neritic") and the average of the open ocean quotas (indicated by and the letter O for "oceanic"), along with the Redfield ratios (R) for the major nutrients. As can be seen, the field quotas for the major nutrients and for the major cations fall well within the range of the laboratory data, except for Mg, the field quotas of which are significantly higher than the laboratory values. The reason for the discrepancy in Mg quotas is unknown but may have to do with the very high concentration of this metal in seawater and differences in washing procedures between the laboratory and field studies.

With the notable exception of Mn, the field quotas for trace elements also fall within the range of laboratory values. The range of quotas observed in the laboratory can thus be taken as a first approximation of the range expected for the trace element composition of phytoplankton in the sea. Moreover, because the coastal and open ocean quotas effectively bracket the average of the laboratory values, the average composition of our laboratory cultures provides an approximate extension of the Redfield formula for the elemental composition of marine phytoplankton. This formula extends further and, perhaps, refines earlier extensions of Redfield by

TABLE 4. Comparison of elemental composition of natural plankton assemblages and suspended particulate materials collected from the field (coastal and open oceans) with the results of this study.

Element	Quota to P	Dissolved metal concentration, Pacific deep water[a]	Martin and Knauer 1973 ($n = 4$)[b], Coastal upwelling water	Martin et al. 1976 ($n = 6$)[c], Oligotrophic open ocean water	Collier and Edmond 1984 ($n = 2$)[d], Oligotrophic open ocean water	Kuss and Kremling 1999 ($n = 9$)[e], Oligotrophic open ocean water	This study ($n = 15$) Nutrient replete culture
K	mol/mol	10 mM	1.5 (0.22)	0.91 (0.27)	N.A.	N.A.	1.7 (1.4)
Mg	mol/mol	54 mM	2.8 (N.A.)	1.4 (0.31)	N.A.	N.A.	0.56 (0.58)
Ca	mol/mol	10 mM	0.65 (0.01)	1.8 (0.68)	5.1 (0.1)	5.4 (2.1)	0.52 (0.51)[f]
Sr	mmol/mol	92 μM	6.4 (0.6)	247 (111)	N.A.	N.A.	5.0 (7.4)[f]
Fe	mmol/mol	0.8–1.0 nM	7.4 (5.5)	3.6 (1.32)	4.6 (0.66)	4.6 (1.3)	7.5 (5.3)
Mn	mmol/mol	0.5–1.0 nM	0.39 (0.21)	0.36 (0.11)	0.34 (0.04)	1.6 (0.18)	3.8 (2.4)
Zn	mmol/mol	8 nM	0.86 (0.63)	1.86 (1.18)	3.03 (1.27)	1.9 (0.69)	0.80 (0.52)
Cu	mmol/mol	2–5 nM	0.18 (0.10)	0.38 (0.06)	0.52 (0.05)	0.37 (0.058)	0.38 (0.35)
Co	mmol/mol	20–30 pM	N.A.	N.A.	N.A.	0.19 (0.021)	0.19 (0.13)
Cd	mmol/mol	0.8–1.0 nM	0.068 (0.016)	0.53 (0.08)	0.54 (0.10)	0.51 (0.085)	0.21 (0.22)
Mo	mmol/mol	100 nM	N.A.	N.A.	N.A.	N.A.	0.03 (0.03)

All particulate metal concentrations are quotas normalized to P. Numbers in the parentheses represent one SD. Dissolved metal concentrations in the Pacific deepwater are given in the third coloumn for reference.

[a]References: K, Mg, Ca, and Sr (Culkin and Cox 1966); Fe (Martin and Gordon 1988); Mn (Landing and Bruland 1980); Zn, Cu, and Cd (Bruland 1980); Co (Knauer et al. 1982).

[b]Data are obtained from the group I raw data in Table 1 of Martin and Knauer (1973). Samples were collected in Monterey Bay, California, with 76 μm aperture phytoplankton net during blooming condition. Only data with Al content less than 100 μg/g (dry weight) are included. High Al concentration in the samples indicates that the data may be biased by the presence of alumino-silicate minerals (Bruland et al. 1991). The phosphorus concentration was obtained from Bruland et al. (1991).

[c]Raw data from Table 7-3 in Martin et al. (1976). Samples were collected in the oligotrophic water of North Pacific open ocean with 64 μm aperture plankton net. Only data with Al less than 100 μg/g are included, i.e. data from Station 69, 73, 75, 77, 78, and 85. The data of Station 54, 81, and 88 are not included as P concentrations were too low (81, 88) or some elements (esp. Fe) were abnormally high (54).

[d]Data from Table 3 in Collier and Edmond (1984). Samples were collected in the open ocean of North Pacific with 44 μm aperture net. Only data of MANOP C (Tow 1 and Tow 2) in the table are included. Other samples had either high Al contents (> 100 μg/g) or no Al data reported.

[e]Suspended particulate matters were collected in the surface water of the Atlantic open ocean with pump and large volume centrifuge system.

[f]The Ca and Sr average quotas do not include the concentrations of the three species (*T. heimii*, *E. huxleyi*, and *G. oceanica*).

Morel and Hudson (1985) based on scant data and by Bruland et al. (1991) based on the three older data sets in Table 4. The unweighted mean of the quotas in Table 2 yields the following formulae. For whole cells, including hard parts (and omitting Mn),

$$(C_{147}N_{16}P_1S_{1.3}K_{1.7}Mg_{0.56}Ca_{23})_{1000}$$
$$Sr_{54}Fe_{7.5}Zn_{0.80}Cu_{0.38}Co_{0.19}Cd_{0.21}Mo_{0.03}$$

And for organic biomass (C quotas after the samples were fumed with HCl and not including the high Ca and Sr quotas of the three $CaCO_3$-forming species, *T. heimii*, *E. huxleyi*, and *G. oceanica*),

$$(C_{124}N_{16}P_1S_{1.3}K_{1.7}Mg_{0.56}Ca_{0.5})_{1000}$$
$$Sr_{5.0}Fe_{7.5}Zn_{0.80}Cu_{0.38}Co_{0.19}Cd_{0.21}Mo_{0.03}$$

A simultaneous examination of the various elemental quotas measured in the field and the average quotas measured in the laboratory (Table 4) gives insight into the variability of phytoplankton composition in seawater. The few data available for K and Mg seem to indicate higher concentrations in neritic compared with oceanic phytoplankton. This is consistent with the high K and Mg quotas we measured in diatoms (Fig. 4) because the coastal samples were apparently collected during a diatom bloom (Bruland et al. 1991). Conversely, the open ocean data show high quotas for Ca and, in a lone sample, a very high quota for Sr. These results are probably due to the presence of calcite precipitating organisms in the samples because high Ca and Sr quotas were also measured in the cultures of the three coccolithforming species, including the hard parts.

Unsurprisingly, because our culture isolates are dominated by coastal species and we used a relatively high Fe concentration in the culture medium, our average Fe quota is similar to the reported coastal value. More surprising are the consistently high Fe quotas also reported for the open ocean samples. It may be that field samples include a sizable fraction of particulate Fe that is not (intra)cellular. For example, Tovar-Sanchez et al. (2003) reported that an average of 47% of the nonlithogenic particulate Fe in southern ocean samples could be dissolved via an oxalate treatment and was thus not "intracellular." These authors reported corrected Fe quotas between 1 and 4 mmol/mol (recalculated using a C:P ratio of 103), in accord with the data of Sunda and Huntsman (1995a) for cultured oceanic phytoplankton. Such low quotas are also in accord with our data for open ocean species (Table 2) and with the open ocean data of Table 4 if these are decreased by a factor of 2.

The disagreement in the Mn quotas between the laboratory and the field are a bit perplexing. In retrospect, the chosen unchelated Mn concentration in our growth medium, $Mn' = 10$ nM, must have been too high. As noted earlier, this Mn′, which is typical of coastal waters (and about 10 times larger than in the open ocean) was chosen to not limit the growth of coastal species. But even the Mn quota from the coastal sample is about 10 times lower than our laboratory average (which is based on rather tightly clustered data; Fig. 3) and is consistent with open ocean data. It may be that this discrepancy comes from a combination of high Mn′ and low Cu′ and Zn′ in our cultures: The Mn quotas of coastal species may be normally kept low by competitive inhibition of Mn uptake by high ambient Cu′ and Zn′ (Sunda and Huntsman 1983, 1996) and that of the open ocean species by low ambient Mn′.

For the remaining trace elements, the most noteworthy features of Table 4 are the high quotas of Co and, particularly, Cd in the open ocean samples. The lone Co quota reported for the open ocean is a perfect match for our average laboratory value. The open ocean Cd quotas are remarkably consistent with each other and much larger than the reported coastal value. These high values for the oceanic Cd quotas, which are higher than all but two of those measured in our individual cultures, presumably reflect extensive substitution of Cd for Zn in the species that live in the Zn-poor open ocean.

In conclusion, it appears that the range and the average of the composition of marine phytoplankton measured in the laboratory—including both major and trace nutrients—are consistent with the composition of the planktonic biomass in the field. Further, the differences in phytoplankton composition that result from variations in both intrinsic physiological properties of individual species and in medium composition (particularly as a result of trace metal interactions and replacement), appear also to be reflected in the differences between the composition of coastal and open ocean samples.

This work was supported by the National Science Foundation Biocomplexity Program (DEB-0083566), the Center for Environmental Bioinorganic Chemistry (CHE-9810248) at the Princeton Environmental Institute at Princeton University, and the National Science Foundation Biocomplexity Program (OCE-0084032) at Rutgers University. We thank Charlotte Fuller (Institute of Marine and Coastal Sciences, Rutgers University) for the elemental analysis for carbon and nitrogen samples. We thank Robert Guillard for providing the provenance information of the coccolithophores (ASM1 and JS1A). We also thank Mak Saito, John Reinfelder, and Oscar Schofield for their help in preparing this manuscript.

Anderson, M. A., Morel, F. M. M. & Guillard, R. R. L. 1978. Growth limitation of a coastal diatom by low zinc ion activity. *Nature* 276:70–1.

Boyd, P. W., Watson, A. J., Law, C. S., Abraham, E. R., Trull, T., Murdoch, R., Bakker, D. C. E., Bowie, A. R., Buesseler, K. O., Chang, H., Charette, M., Croot, P., Downing, K., Frew, R., Gall, M., Hadfield, M., Hall, J., Harvey, M., Jameson, G., LaRoche, J., Liddicoat, M., Ling, R., Maldonado, M. T., McKay, R. M., Nodder, S., Pickmere, S., Pridmore, R., Rintoul, S., Safi, K., Sutton, P., Strzepek, R., Tanneberger, K., Turner, S., Waite, A. & Zeldis, J. 2000. A mesoscale phytoplankton bloom in the polar southern ocean stimulated by Fe fertilization. *Nature* 407:695–702.

Brand, L. E. 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. *Limnol. Oceanogr.* 36:1756–71.

Brand, L. E., Sunda, W. G. & Guillard, R. R. L. 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28:1182–98.

Brand, L. E., Sunda, W. G. & Guillard, R. R. L. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96:225–50.

Bruland, K. W. 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. *Earth Planet. Sci. Lett.* 47:176–98.

Bruland, K. W. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 34:269–85.

Bruland, K. W. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 37: 1008–17.

Bruland, K. W., Donat, J. R. & Hutchins, D. A. 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* 36:1555–77.

Coale, K. H., Fitzwater, S. E., Gordon, R. M., Johnson, K. S. & Barber, R. T. 1996. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. *Nature* 379:621–4.

Collier, R. & Edmond, J. 1984. The trace element geochemistry of marine biogenic particulate matter. *Progr. Oceanogr.* 13: 113–99.

Culkin, F. & Cox, R. A. 1966. Sodium, potassium, magnesium, calcium and strontium in sea water. *Deep-Sea Res.* 13:789–804.

Cullen, J. T. & Sherrell, R. M. 1999. Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California. *Mar. Chem.* 67:233–47.

Ellwood, M. J. & Hunter, K. A. 2000. The incorporation of zinc and iron into the frustule of the marine diatom *Thalassiosira pseudonana*. *Limnol. Oceanogr.* 45:1517–24.

Falkowski, P. G. 2000. Rationalizing elemental ratios in unicellular algae. *J. Phycol.* 36:3–6.

Falkowski, P. G. & Raven, J. A. 1997. *Aquatic Photosynthesis*. Blackwell Science, Malden, MA, 375 pp.

Fleming, R. H. 1940. The composition of plankton and units for reporting populations and production. *Pacific Sci. Congr. Calif. Proc.*, 6th, 1939. 3:535–40.

Geider, R. J. & La Roche, J. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37:1–17.

Granger, J. & Price, N. M. 1999. The importance of siderophores in iron nutrition of heterotrophic marine bacteria. *Limnol. Oceanogr.* 44:541–55.

Harrison, G. I. & Morel, F. M. M. 1986. Response of the marine diatom *Thalassiosira weissflogii* to iron stress. *Limnol. Oceanogr.* 31:989–97.

Hudson, R. J. M. & Morel, F. M. M. 1989. Distinguishing between extracellular and intracellular iron in marine phytoplankton. *Limnol. Oceanogr.* 34:1113–20.

Hudson, R. J. M. & Morel, F. M. M. 1990. Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions. *Limnol. Oceanogr.* 35:1002–20.

Hutchins, D. A., Witter, A. E., Butler, A. & Luther, G. W. 1999. Competition among marine phytoplankton for different chelated iron species. *Nature* 400:858–61.

Knauer, G. A., Martin, J. H. & Gordon, R. M. 1982. Cobalt in northeast pacific waters. *Nature* 297:49–51.

Kolber, Z. S., Prasil, O. & Falkowski, P. G. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochim Biophys Acta Bioenerg.* 1367:88–106.

Kuss, J. & Kremling, K. 1999. Spatial variability of particle associated trace elements in near-surface waters of the North Atlantic (30 degrees N/60 degrees W to 60 degrees N/ 2 degrees W), derived by large-volume sampling. *Mar. Chem.* 68:71–86.

Landing, W. M. & Bruland, K. W. 1980. Manganese in the north Pacific. *Earth Planet. Sci. Lett.* 49:45–56.

Lee, J. G. & Morel, F. M. M. 1995. Replacement of zinc by cadmium in marine phytoplankton. *Mar. Ecol. Prog. Ser.* 127:305–9.

Lee, J. G., Roberts, S. B. & Morel, F. M. M. 1995. Cadmium: a nutrient for the marine diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 40:1056–63.

Maldonado, M. T. & Price, N. M. 1996. Influence of N substrate on Fe requirements of marine centric diatoms. *Mar. Ecol. Prog. Ser.* 141:161–72.

Maldonado, M. T. & Price, N. M. 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. *Deep-Sea. Res. II* 46:2447–73.

Martin, J. H., Bruland, K. W. & Broenkow, W. W. 1976. Cadmium transport in the California current. *In* Windom, H. L. & Duce, R. A. [Eds.] *Marine Pollutant Transfer*. Lexington Books (D. C. Health and Co., Toronto), pp. 159–84.

Martin, J. H., Coale, K. H., Johnson, K. S., Fitzwater, S. E., Gordon, R. M., Tanner, S. J., Hunter, C. N., Elrod, V. A., Nowicki, J. L., Coley, T. L., Barber, R. T., Lindley, S., Watson, A. J., Van Scoy, K., Law, C. S., Liddicoat, M. I., Ling, R., Stanton, T., Stockel, J., Collins, C., Anderson, A., Bidigare, R., Ondrusek, M., Latasa, M., Millero, F. J., Lee, K., Yao, W., Zhang, J. -Z., Friederich, G., Sakamoto, C., Chavez, F., Buck, K., Kolber, Z., Greene, R., Falkowski, P. G., Chisholm, S. W., Hoge, F., Swift, R., Yungel, J., Turner, S., Nightingale, P., Hatton, A., Liss, P. & Tindale, N. W. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature* 371:123–9.

Martin, J. H. & Fitzwater, S. E. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* 331:341–3.

Martin, J. H. & Gordon, R. M. 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. *Deep-Sea Res. I* 35:177–96.

Martin, J. H. & Knauer, G. A. 1973. The elemental composition of plankton. *Geochim. Cosmochim. Acta* 37:1639–53.

Moffett, J. W. 1995. The spatial and temporal variability of copper complexation by strong organic ligands in the Sargasso Sea. *Deep-Sea Res. I* 42:1273–95.

Morel, F. M. M. & Hudson, R. J. M. 1985. The geobiological cycle of trace elements in aquatic systems: Redfield revisited. *In* Strumm, W. [ed.] *Chemical Processes in Lakes*, John Wiley, New York, p 251–81.

Morel, F. M. M. & Hering, J. G. 1993. *Principles and Applications of Aquatic Chemistry*. Wiley, New York, 374 pp.

Morel, F. M. M., Reinfelder, J. R., Roberts, S. B., Chamberlain, C. P., Lee, J. G. & Yee, D. 1994. Zinc and carbon co-limitation of marine-phytoplankton. *Nature* 369:740–2.

Outten, C. E. & O'Halloran, T. V. 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science* 292:2488–92.

Payne, C. D. & Price, N. M. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. *J. Phycol.* 35:293–302.

Price, N. M., Andersen, L. F. & Morel F. M. M. 1991. Iron and nitrogen nutrition of equatorial pacific plankton. *Deep-Sea Res. I* 11:1361–78.

Price, N. M., Harrison, G. I., Hering, J. G., Hudson, R. J., Nirel, P. M. V., Palenik, B. & Morel, F. M. M. 1988/1989. Preparation and chemistry of the artificial algal culture medium Aquil. *Biol. Oceanogr.* 6:443–61.

Price, N. M. & Morel, F. M. M. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. *Nature* 344: 658–60.

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., Schofield, O., Morel, F. M. M. & Falkowski, P. G. 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, 425:291–4.

Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *In* Daniel, R. J. [Ed.] James Johnstone Memorial Volume. Liverpool University Press, pp. 176–92.

Redfield, A. C. 1958. The biological control of the chemical factors in the environment. *Am. Sci.* 46:205–21.

Redfield, A. C., Ketchum, B. H. & Richards, F. A. 1963. The influence of organisms on the composition of sea-water. *In* Hill, M. N. [Ed.] *The Sea*. Interscience Publication, New York pp. 26–77.

Rue, E. L. & Bruland, K. W. 1995. Complexation of iron III by natural organic ligands in the Central north Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Mar. Chem.* 50:117–38.

Saito, M. A. & Moffett, J. W. 2001. Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. *Mar. Chem.* 75:49–68.

Saito, M. A., Moffett, J. W., Chisholm, S. W. & Waterbury, J. B. 2002. Cobalt limitation and uptake in *Prochlorococcus*. *Limnol. Oceanogr.* 47:1629–36.

Sambrotto, R. N., Savidge, G., Robinson, C., Boyd, P., Takahashi, T., Karl, D. M., Langdon, C., Chipman, D., Marra, J. & Codispoti, L. 1993. Elevated consumption of carbon relative to nitrogen in the surface ocean. *Nature* 363:248–50.

Stoll, H. M., Rosenthal, Y. & Falkowski, P. G. 2002. Climate proxies from Sr/Ca of coccolith calcite: calibrations from continuous culture of *Emiliania huxleyi*. *Geochim. Cosmochim. Acta* 66:927–36.

Sunda, W. G. & Huntsman, S. A. 1983. Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. *Limnol. Oceanogr.* 28:924–34.

Sunda, W. G. & Huntsman, S. A. 1992. Feedback interactions between zinc and phytoplankton in seawater. *Limnol. Oceanogr.* 37:25–40.

Sunda, W. G. & Huntsman, S. A. 1995a. Iron uptake and growth limitation in oceanic and coastal phytoplankton. *Mar. Chem.* 50:189–206.

Sunda, W. G. & Huntsman, S. A. 1995b. Cobalt and zinc interreplacement in marine phytoplankton: biological and geochemical implications. *Limnol. Oceanogr.* 40:1404–17.

Sunda, W. G. & Huntsman, S. A. 1995c. Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnol. Oceanogr.* 40:132–7.

Sunda, W. G. & Huntsman, S. A. 1996. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol. Oceanogr.* 41:373–87.

Sunda, W. G. & Huntsman, S. A. 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* 390:389–92.

Sunda, W. G. & Huntsman, S. A. 1998a. Interactions among Cu^{2+}, Zn^{2+}, and Mn^{2+} in controlling cellular Mn, Zn, and growth rate in the coastal alga Chlamydomonas. *Limnol. Oceanogr.* 43:1055–64.

Sunda, W. G. & Huntsman, S. A. 1998b. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater. *Environ. Sci. Technol.* 32:2961–68.

Sunda, W. G. & Huntsman, S. A. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. *Limnol. Oceanogr.* 45:1501–16.

Tovar-Sanchez, A., Sañudo-Wilhelmy, S. A., Garcia-Vargas, M., Weaver, R. S., Popels, L. C. & Hutchins, D. A. 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. *Mar. Chem.* 82:91–9.

Westall, J. C., Zachary, J. L. & Morel, F. M. M. 1986. *MINEQL: A Computer Program for the Calculation of the Chemical Equilibrium Composition of Aqueous Systems*. Report 86-01, Department of Chemistry, Oregon State University, Corvallis, Oregon, 91 pp.

Yee, D. & Morel, F. M. M. 1996. In vivo substitution of zinc by cobalt in carbonic anhydrase of a marine diatom. *Limnol. Oceanogr.* 41:573–7.