

Electronic Supplementary Material

M1 - Materials and Methods

Statistical analyses using the combined data set.

In addition to the details provided in the materials and methods section in the test, the authors also considered the following when conducting the statistical analyses on the combined data set.

To test hypotheses on the evolutionary inheritance of stoichiometry in phytoplankton, we combined the findings from the present study with those in an earlier study (Quigg et al. 2003; Ho et al. 2003) (Table 1). This gave us a large data set (87 observations) and diversity of hierarchical levels (4 lineages, 29 species) to test the hypotheses. Of the 29 species examined, most had 3 or 4 observations of elemental composition, but two species had only 2 observations and several species had more than 4 observations. To balance the sampling design for statistical tests, species with >3 replicates had excess replicates removed at random and for the two species with only two observations an average of the two observations was used as an estimate of a third replicate. We considered Fe, Mn, Zn, Cu, Co, and Cd in the statistical analyses because taxonomic differences in these elemental ratios have been shown to be associated with the plastid (Quigg et al. 2003). The Glaucocystophyta are represented by one species and so their variability is likely under sampled compared to that of other groups. Similarly, caution is necessary when interpreting the differences between groups with only one species or a small number of observations such as the Euglenophyceae and Cryptophyceae.

Trees were generated using divisive hierarchical clustering (R Development Core Team; 2008; Diana algorithm in package cluster), omitting GLA and RG2 as these classes have only one species each and their position in the tree is likely to be more uncertain. The algorithm begins with all observations in one cluster and creates divisions by selecting the cluster containing the most dissimilar observations and starting a new cluster with the observation with the largest mean dissimilarity to other observations in the cluster. Observations closer to the new cluster than to the old cluster were moved to the new cluster. Clusters were divided until each cluster contained only a single observation. To test the robustness of the tree, we performed a cross-validation analysis omitting one of the 87 observations at a time, and compared the trees. We

checked our tree using agglomerative algorithms with several different agglomeration methods (complete, single, Ward's and average) using two algorithms ("hclust" and "agnes" in R). All trees had the same topology for C, R1, R2, G1, and G2, although branch lengths changed slightly. We performed a similar analysis on the larger set of categories (C, GLA, R1, R2, RG2, G1, G2) and found that the tree varied somewhat with the algorithm. Therefore we decided to show only the tree with the 5 largest categories. The statistical significance of the distances between groups, that included more than one species, due to trace element composition were estimated by bootstrapping omitting one species at a time for 1000 replicate calculations.

Ho, T-Y., Quigg, A., Finkel, Z. V., Milligan, A., Wyman, K., Falkowski, P. G. & Morel, F. M. M. 2003 On the elemental composition of some marine phytoplankton. *J. Phycol.* **39**, 1-15.

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**, 291–294.

R Development Core Team. 2008 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

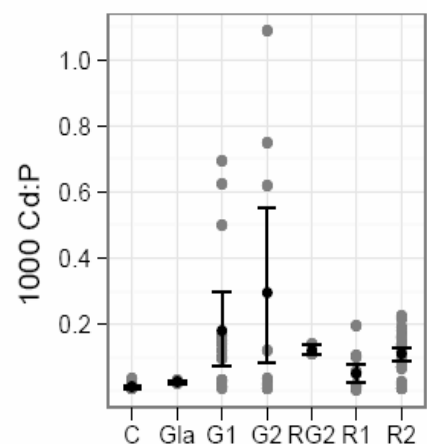
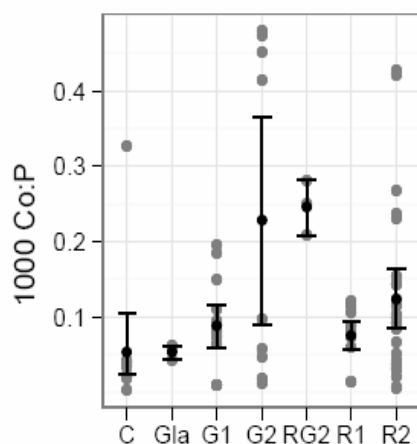
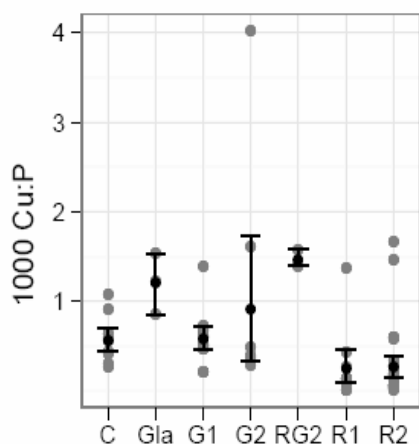
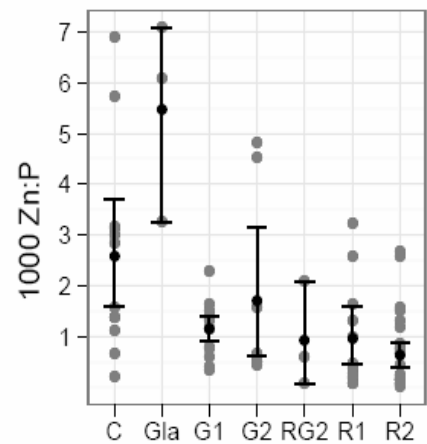
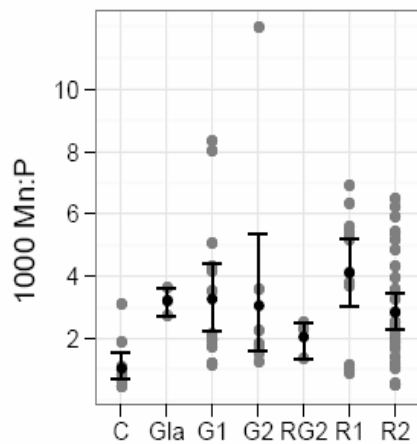
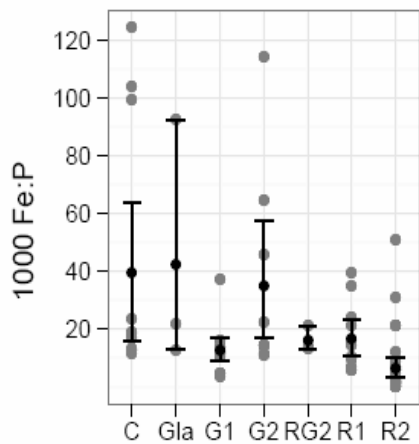
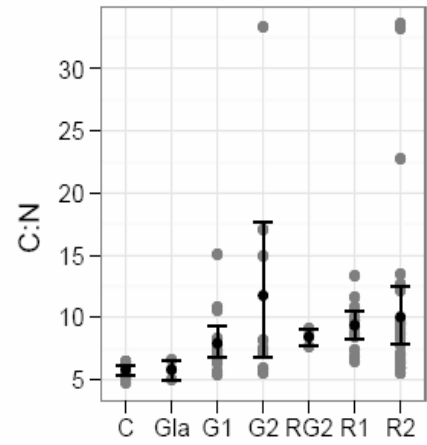
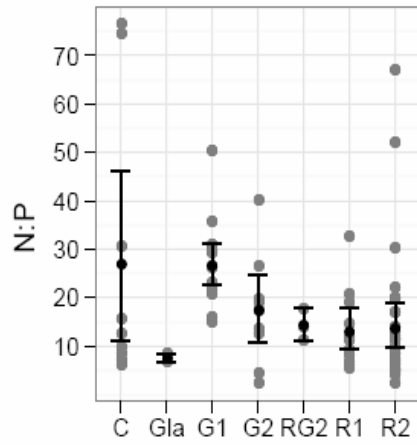
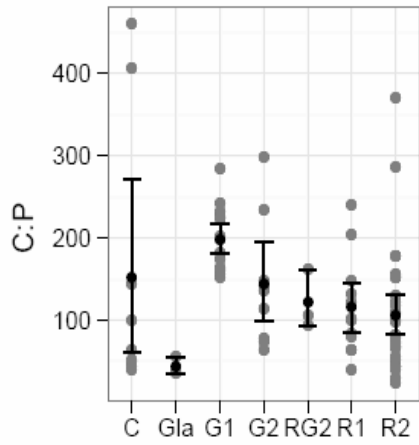
Quigg, Irwin, Finkel
Evolutionary inheritance of elemental stoichiometry in phytoplankton.
Proceedings of the Royal Society. B. Biological Sciences.

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Figure 1

Phytoplankton cellular element:P ratios for the combined datasets.

C and N have units of mol:mol P while Fe, Mn, Zn, Cu, Co, and Cd have units of mmol:mol P. C:N ratio has units of mol:mol. Elemental ratios were grouped by lineage and major endosymbiotic event for macronutrients. Species and replicates are shown as grey dots. The mean ratio and bootstrap 95% confidence interval on the mean is shown as a black symbol and error bars.



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Table S1 Phytoplankton species investigated.

Statistical analyses were performed on phytoplankton from the current study as well as those from a previous study (Quigg *et al.* 2003; Ho *et al.* 2003) using lineage, group, endosymbiotic event and species as groupings. Phytoplankton strains cultured in the present study and their specific growth rate (d^{-1}) are also included.

Culture Collection of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA and,

University of Texas, Culture Collection of Algae (UTEX), Austin, TX, USA.

^ Cultures provided by Dr. John Waterbury (Woods Hole Oceanographic institution, USA).

Cultures provided by Dr. Ian Probert (Algobank, Universite de Caen Basse Normandie, France).

Lineages	Classes	Event	Species	Previous study	Current study	Specific growth rate (d ⁻¹)	
Cyanophyta (C)	Cyanophyceae	C	<i>Synechococcus</i> sp.		CCMP835	0.67	
		C	<i>Cyanothece</i> sp.		WH8904^	0.33	
		C	<i>Trichodesmium</i> sp.		IMS101^	0.25	
		C	<i>Anabaena flos-aquae</i>		UTEXLB2557	0.61	
Glaucocystophyta (GLA)	Glaucocystophyceae	GLA	<i>Cyanophora paradoxa</i>		UTEXLB555	0.23	
Green plastid lineage (G)	Prasinophyceae	G1	<i>Pyramimonas parkeae</i>	*			
		G1	<i>Tetraselmis</i> sp.	*			
		G1	<i>Pycnococcus provasoli</i>	*			
	Chlorophyceae	G1	<i>Dunaliella tertiolecta</i>	*			
		G1	<i>Nannochloris atomus</i>	*			
	Chlorarachniophyceae	G2	<i>Chlorarachnion globosum</i>		AC132#	0.73	
		G2	<i>Chlorarachnion reptans</i>		AC133#	0.41	
	Red plastid lineage (R)	Euglenophyceae	G2	<i>Eutreptiella</i> sp.		CCMP389	0.35
		Rhodophyceae	R1	<i>Rhodella maculata</i>		AC123#	0.38
			R1	<i>Rhodorus marinus</i>		(R16)	0.39
Chrysophyceae		R1	<i>Porphyridium aerugineum</i>		UTEXLB755	0.24	
		R1	<i>Porphyridium purpureum</i>		AC120#	1.11	
Cryptophyceae		R2	<i>Ochromonas</i> sp.		CCMP1278	0.35	
		R2	<i>Rhodomonas salina</i>		CCMP1319	0.86	
Haptophyceae		R2	<i>Emiliania huxleyi</i>	*			
		R2	<i>Gephyrocapsa oceanica</i>	*			
Bacillariophyceae		R2	<i>Ditylum brightwellii</i>	*			
	R2	<i>Thalassiosira eccentrica</i>	*				
	R2	<i>Thalassiosira weissflogii</i>	*				
	R2	<i>Nitzschia brevirostris</i>	*				
Dinophyceae	R2	<i>Prorocentrum minimum</i>	*				
	R2	<i>Amphidinium carterae</i>	*				
	R2	<i>Thoracosphaera heimii</i>	*				
	RG2	<i>Gymnodinium chlorophorum</i>	*				

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Table S2

Elemental composition of 14 species of phytoplankton investigated in this present study.

Units of element:P for C, N, P, S, K, Mg, and Ca are mol:mol while the units for the other elements (Sr, Fe, Mn, Zn, Cu, Co, Cd, and Mo) are mmol:mol. Errors are standard errors of $n \geq 3$ samples measured; each sample was taken from a separate culture. (nd = not determined)

Species	C	N	S	K	Mg	Ca	Sr	Fe	Mn	Zn	Cu	Co	Cd	Mo
<i>Synechococcus</i> sp.	52 ±9.8	8.7 ±1.8	1.3 ±0.12	1.0 ±0.3	1.2 ±0.3	0.24 ±0.05	0.52 ±0.16	16.8 ±3.4	2.4 ±0.33	1.1 ±0.19	0.65 ±0.06	0.086 ±0.05	0.02 ±0.004	0.01 ±0.003
<i>Cyanotheca</i> sp.	61 ±5.8	11.7 ±1.0	nd	nd	nd	nd	nd	13.1 ±1.0	0.54 ±0.03	2.34 ±0.27	0.48 ±0.03	0.02 ±0.0005	0.008 ±0.001	0.6 ±0.08
<i>Trichodesmium</i> sp.	161 ±12	31 ±0.82	0.19 ±0.06	0.30 ±0.06	0.07 ±0.01	94 ±5	107 ±4	109 ±6	0.93 ±0.08	3.9 ±0.93	0.8 ±0.09	0.03 ±0.005	0.006 ±0.001	0.18 ±0.005
<i>Anabaena flos-</i> <i>aquae</i>	156 ±11	50 ±5	0.74 ±0.26	nd	nd	nd	nd	19 ±1.3	0.77 ±0.06	2 ±0.78	0.35 ±0.03	0.04 ±0.001	0.005 ±0.0004	0.10 ±0.01
<i>Chlorarachnion</i> <i>globosum</i>	111 ±17	19 ±3.3	0.37 ±0.001	0.51 ±0.18	0.37 ±0.13	0.11 ±0.01	1.91 ±0.59	16.1 ±2.8	2.2 ±0.6	0.9 ±0.26	0.83 ±0.32	0.02 ±0.009	0.01 ±0.003	0.02 ±0.005
<i>Chlorarachnion</i> <i>reptans</i>	70 ±5	3.3 ±0.7	0.50 ±0.06	0.48 ±0.01	3.8 ±0.64	3.2 ±0.85	237 ±34	55 ±6.6	2.0 ±0.15	4.7 ±0.11	0.3 ±0.001	0.08 ±0.01	0.03 ±0.005	0.04 ±0.02
<i>Eutreptiella</i> sp.	144 ±5	19 ±1	0.13 ±0.03	0.72 ±0.03	0.07 ±0.005	0.07 ±0.005	1.56 ±0.32	14 ±0.9	1.6 ±0.05	0.59 ±0.09	0.36 ±0.03	0.46 ±0.02	0.93 ±0.16	0.026 ±0.009
<i>Cyanophora</i> <i>paradoxa</i>	43 ±4	7.5 ±0.37	1.2 ±0.09	0.05 ±0.03	0.25 ±0.07	0.21 ±0.02	0.033 ±0.001	19 ±2.7	3.3 ±0.2	5 ±0.8	1.2 ±0.12	0.05 ±0.004	0.02 ±0.002	0.03 ±0.005
<i>Rhodella</i> <i>maculata</i>	176 ±13	15 ±2.3	0.09 ±0.01	0.79 ±0.08	0.72 ±0.02	0.43 ±0.15	2.5 ±0.42	17 ±1.4	5.9 ±0.6	1.7 ±0.29	0.4 ±0.06	0.09 ±0.006	0.06 ±0.003	0.02 ±0.004
<i>Rhodorus</i> <i>marinus</i>	77 ±10	7.9 ±1.5	1.13 ±0.03	0.24 ±0.07	0.65 ±0.10	0.34 ±0.25	1.88 ±1.33	8.4 ±1.3	5.3 ±0.07	0.27 ±0.09	0.11 ±0.01	0.09 ±0.01	0.07 ±0.02	0.02 ±0.008
<i>Porphyridium</i> <i>aerugineum</i>	111 ±9	11 ±1.4	1.0 ±0.12	0.21 ±0.07	0.38 ±0.11	0.20 ±0.06	0.12 ±0.02	33 ±4	4.5 ±0.45	1.6 ±0.72	0.13 ±0.002	0.08 ±0.01	0.07 ±0.05	0.02 ±0.003
<i>Porphyridium</i> <i>purpureum</i>	113 ±7	12 ±2.8	0.29 ±0.06	0.64 ±0.12	0.59 ±0.15	0.33 ±0.06	3.36 ±0.68	10.4 ±2.1	1.0 ±0.04	0.3 ±0.06	0.08 ±0.03	0.04 ±0.02	0.01 ±0.003	0.01 ±0.0005
<i>Ochromonas</i> sp.	120 ±17	20 ±3	0.31 ±0.002	0.18 ±0.04	0.34 ±0.06	0.13 ±0.014	2.56 ±0.33	27 ±7	3 ±0.4	1.75 ±0.27	0.5 ±0.07	0.03 ±0.002	0.005 ±0.001	0.008 ±0.001
<i>Rhodomonas</i> <i>salina</i>	294 ±22	53 ±5	2.1 ±0.16	0.18 ±0.06	1.33 ±0.24	0.29 ±0.06	1.3 ±0.17	21 ±4.6	2.5 ±0.32	1.6 ±0.5	1.3 ±0.16	0.05 ±0.002	0.19 ±0.01	0.05 ±0.03
Average	130	20	0.75	0.43	0.83	10.5	40	26	2.5	1.9	0.6	0.09	0.1	0.1
RSD (%)	10	12	14	15	19	42	46	13	11	12	13	18	31	25

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Table S3 Statistical analyses performed on full (29 species) dataset.

A. Pair-wise t-tests for significant differences in elemental ratios between Cyanophyta (C), Glaucocystophyta (GLA), and the green (G) and red (R) plastid lineages.

All phytoplankton examined in the present and previous study (Quigg et al. 2003) were included in this analysis with 3 replicates per species (N=87). *G. chlorophorum* is included with the green lineage in this analysis as it has a green plastid. Each column reports the group for which the log element:P ratio is significantly different. The significance level ($p \leq 0.05$) was adjusted for multiple comparisons with Holm's correction.

Log (Element:P)	C	GLA	G	R
Log C:P		G,R	GLA,R	GLA,G
Log N:P		G,R	GLA,R	GLA,G
Log Fe:P	R		R	C,G
Log Mn:P	GLA,G,R	C	C	C
Log Zn:P	GLA,R	C,G,R	GLA,R	C,GLA,G
Log Cu:P	R	R	R	C,GLA,G
Log Co:P				
Log Cd:P	GLA,G,R	C,G,R	C,GLA	C,GLA

B. Distance matrix for the average log trace element:P profiles in Cyanophyta (C), Glaucocystophyta (GLA), the green (G1, G2) and red lineages (R1, R2), and the green dinoflagellate (RG2).

All phytoplankton examined in the present and a previous study (Quigg et al. 2003), were included in this analysis with 3 replicates per species. Distances have been scaled to a maximum of 1.0. Due to the small sample sizes in many of these categories, the 95% bootstrapped confidence intervals of many of these distances overlap, see text for more detail.

	C	GLA	G1	G2	RG2	R1	R2
C		0.70	0.74	0.73	0.99	0.80	1.00
GLA	0.70		0.50	0.49	0.79	0.70	0.98
G1	0.74	0.50		0.27	0.52	0.45	0.52
G2	0.73	0.49	0.27		0.40	0.50	0.67
RG2	0.99	0.79	0.52	0.40		0.78	0.75
R1	0.80	0.70	0.45	0.50	0.78		0.52
R2	1.00	0.98	0.52	0.67	0.75	0.52	

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