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A first look at paleotemperature prospects from Mg in coccolith carbonate: Cleaning techniques and culture measurements

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[1] **Abstract:** Although coccolith calcite is abundant in carbonate sediments, it has not been previously utilized for Mg/Ca paleothermometry. Cleaning experiments with synthetic composite samples (reagent $CaCO_3$ powder and organic matter from noncalcifying marine algae *Chlorella*) are used to evaluate which traditional and/or novel cleaning methods permit us to recover the known carbonate Mg/Ca ratio. The most effective cleaning treatment, causing the least effect on carbonate chemistry and most complete and rapid oxidation of algal organic matter, was oxidation in an equal volume mixture of sodium hypochlorite (2.8%) and hydrogen peroxide (30%). However, in some organic- rich samples this method may not remove sufficient noncarbonate Mg to permit precise determination of carbonate Mg/Ca. Mg/Ca ratios in small culture samples may be determined precisely (relative standard deviation of 0.65%) using sector field inductively coupled plasma-mass spectrometry. Measurements of Mg/Ca in coccoliths from several species suggest that temperature may be an important control over Mg partitioning in coccolith calcite, although cleaning issues are likely to be an important limitation on paleoceanographic application.

Keywords: Mg/Ca; paleothermometry; coccoliths; cleaning techniques; analytical techniques.

Index terms: Paleoceanography; trace elements; geochemistry.

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1. Introduction

[2] While previous applications of Mg/Ca for paleothermometry have focused extensively on foraminifera, recent work with stable isotopes [e.g., *Ziveri et al.*, 2000] and trace elements [*Stoll and Schrag*, 2000; *Rosenthal et al.*, 1999; *Stoll et al.*, 2001] has shown that the abundant calcite produced by coccolithophorids may also be useful for geochemical studies. Coccolith calcite is a natural locus for expanding the range of Mg/Ca thermometry. As in foraminiferal and abiogenic calcites, it is likely that temperature exerts a dominant control over Mg partitioning in coccolith calcite. Paleotemperature estimates derived from coccolith Mg/Ca would complement existing capabilities with foraminiferal Mg/Ca in several ways. The interpretation of several geochemical characteristics of coccolithophorids currently utilized for paleoceanographic studies (including Sr/Ca of coccolith carbonate and carbon isotope fractionation in alkenone biomarkers) requires paleotemperature estimates, and the best estimates would be those derived from the same group of organisms. An existing temperature indicator derived from coccolithophorids, the undersaturation ratio of alkenone biomarkers (U^{k}_{37}), requires relatively large samples for isolation of alkenones and may be biased by ecological or physiological factors [e.g., *Conte et al.*, 1998; *Epstein et al.*, 1998]. Temperature estimates from coccolith Mg/Ca might elucidate some of these latter complications. Finally, coccolith Mg/Ca might be useful for temperature reconstructions in areas where foraminifera are not present or where the foraminiferal Mg/Ca record might be compromised by selective dissolution due to heterogeneous distribution of Mg in foraminifer tests [e.g., *Rosenthal et al.*, 2000; *Brown and Elderfield*, 1996]. Mg may be distributed more homogeneously in coccolith calcite since many identical heterococcoliths are produced continuously during a single life stage.

[3] The utility of carbonate chemistry of any fraction of the sediments depends on the ability to measure the chemistry of the carbonate fraction alone, without contributions from other phases in the sediments. Foraminiferal carbonate can be physically separated from other particles, although various approaches are required to remove overgrowths and absorbed phases for some elements [*Boyle*, 1983; *Boyle and Keigwin*, 1986]. However, most coccoliths range from 2 to 8 μm in diameter and cannot be physically separated from other sediment phases. In addition, samples from culture and sediment traps contain a much higher fraction of organic matter than typical open-ocean sediments. Magnesium is very enriched in the

organic fraction; it is a center metal in pheoporphyrin rings of chlorophyll *a* and is also an essential constituent of the adenosine triphosphate (ATP) molecule and therefore, when released at low pH during carbonate dissolution, can dramatically alter measured "carbonate" elemental ratios. Typical *Emiliania huxleyi* samples from culture experiments contain $5–25 \times 10^{-14}$ g Mg/cell in organic fractions, 100–500 times higher than that of the $CaCO_3$, which contains only 5×10^{-16} g Mg/cell (Y. Rosenthal, unpublished data, 2000). In contrast, organic phases do not appear to be important sources of Sr, and Sr/Ca ratios of coccoliths from cultures are not affected by removal of the organic phase (H. M. Stoll et al., Calibration of coccolith climate proxies: Discerning kinetic and biological effects on Sr partitioning in coccoliths of *Emiliania huxleyi* from continuous culture, manuscript in preparation, 2001).

[4] In this paper, we examine the types of cleaning procedures necessary to eliminate Mg from organic phases in composite organic/carbonate samples (section 2). An inductively coupled plasma-mass spectrometry (ICP-MS) analytical method for precise measurement of coccolith Mg/Ca in very small coccolith samples is described in section 3. Finally, we present the first precise Mg/Ca ratios of coccoliths of *E. huxleyi*, *Gephyrocapsa oceanica*, and *Calcidiscus leptoporus* grown in culture at different temperatures (section 4).

[5] This evaluation of different cleaning methods differs significantly from most previously published comparisons, which are based on comparing the chemistry of biogenic carbonate sample of unknown composition with and without application of various cleaning procedures. However, since the actual chemistry of the carbonate portion of the sample is not known a priori, it is difficult to assess objectively when noncarbonate contributions have been eliminated. We create synthetic samples from reagent-grade $CaCO_3$ powder (similar in chemistry and particle size to coccolith carbonate) of known Mg/Ca ratio and algal organic matter (from noncalcifying marine algae *Chlorella*). With these samples it is possible to evaluate which cleaning procedures permit us to recover the known carbonate Mg/Ca ratio upon dissolution. To our knowledge, this is the first published description of the latter type of experiment for Mg/Ca. These experiments demonstrate that neither cessation of Mg release (or plateau in Mg release) in cleaning/rinse solutions nor reproducibility of Mg/Ca ratios (in the dissolved carbonate) with different types of cleaning treatments are sufficient criteria to assess the complete removal of noncarbonate Mg in carbonate samples.

2. Methods for Cleaning Mg From Organic Phases

2.1. Experiments Comparing Cleaning Efficiency With Synthetic Composite Samples

[6] An effective method for cleaning Mg in marine carbonates must (1) not alter the chemistry of the calcium carbonate phase [e.g., *Love and Woronow*, 1991] and (2) efficiently remove noncarbonate sources of Mg. Replicate samples of 5 mg of reagent $CaCO_3$ (Mg/Ca ratio of 0.4 mmol/mol) were used to evaluate the effect of several different novel and traditional cleaning methods (Table 1) on carbonate Mg/Ca. Replicate composite samples of reagent $CaCO_3$ "contaminated" with *Chlorella* algae were used to evaluate the efficiency of these methods for removal of organic Mg (for details on preparation of composite samples, see Tables 2 and 3). Composite "contaminated" samples had much higher Mg/Ca ratios (Mg/Ca ratios of 33 and 44 mmol/mol for first and second set of

Table 1. Summary of Different Cleaning Methods Employed

Method	Cleaning Solution	Amount per 5 mg $CaCO_3$	Duration	Temperature, °C	Approximate pH of Solution[a]	Reference
H_2O_2 /NaOCl	50% volume H_2O_2 (30%) and 50% volume NaOCl (3%)	10 mL	1 hour[b]	25°	8–9	*Bairbakesh et al.* [1999]
Boyle	0.15% H_2O_2(30%) in 0.1 *N* NaOH	20 mL	30 min	80°	>10	*Boyle* [1983]
H_2O_2	50% volume H_2O_2 (30%) and 50% volume 0.1 *N* NaOH	10 mL	1 hour	50°	>10	
TMAH 5%	5% TMAH in distilled water	2.5 mL	4 hours	80°	9	*Uchida et al.* [1996] (modified)
TMAH 1%	1% TMAH in 50% ethanol	2.5 mL	4 hours	50°	9	*Uchida et al.* [1996] (modified)

[a]The pH of solutions before reaction with organic matter.
[b]Samples subjected to 10 s ultrasonication and addition of 500 μL 3% NaOCl every 20 min.

composite samples, respectively) than the carbonate alone. Mg/Ca ratios of these composite samples fall in the range of Mg/Ca ratios observed for uncleaned samples extracted from coccolithophorid cultures, sediment traps, and sediments with high organic carbon contents (0.5–300 mmol/mol).

[7] A first series of experiments compared five different cleaning methods summarized in Table 1. All use alkaline solutions to minimize dissolution of carbonate on liberation of CO_2 during oxidation of organic matter. Solubilization of organic matter using tetramethlyammoniahydroxide (TMAH) has not been previously used to clean marine carbonate samples although it has been widely used in the preparation of biological and especially botanical samples for elemental analysis [*Uchida et al.*, 1996]. Protocols were modified here since the ethylenediamine tetraacetic acid (EDTA) matrix in published methods rapidly dissolves the carbonate. The bleach/peroxide method was originally proposed to disaggregate fine carbonates in sediment trap samples while avoiding overgrowths which occur on samples treated with oxidizing agents at higher pH [*Bairbakesh et al.*, 1999]. Other methods designed for cleaning carbonates have been modified only slightly. For example, for the Boyle oxidation method [*Boyle*, 1983], because of the large amounts of organic matter in the samples of this study, we used 20 mL of oxidizing agent at 80°C rather than the 0.5–1 mL used typically.

[8] After the cleaning treatments, composite (*Chlorella* plus $CaCO_3$) samples were rinsed 10–15 times in 5 mL of distilled water (pH adjusted to 8.5 with NH_4OH). All cleaning solutions and subsequent rinses were analyzed for Mg and Ca release via flame atomic absorption spectroscopy (AAS) with matrix-matched standards. Carbonate samples were dissolved in 400 μL of 2% HNO_3 and analyzed via flame atomic absorption spectroscopy with matrix-matched standards.

[9] A second series of cleaning experiments was conceived to compare the efficiency of multiple oxidizing steps using the bleach/peroxide oxidation method and also to assess whether ion exchange steps either before or after oxidation would improve the efficiency of Mg removal from organic sources. Table 3 indicates the treatments received by each split and details of the preparation of the composite

Table 2. Effect of Various Oxidizing and TMAH Treatments on Carbonate Chemistry and Removal of Organic Mg

Split	Sample	Method	Mg/Ca on Dissolution, mmol/mol	Cleaning Efficiency[a]	Total Efficiency[b]	Percent Difference Carbonate Mg/Ca
1, 2	$CaCO_3$	H_2O_2/NaOCl	0.386			−1.6%
3, 4	$CaCO_3$	Boyle	0.378			0.2%
5, 6	$CaCO_3$	H_2O_2	0.374			−4.6%
7, 8	$CaCO_3$	TMAH 5%	0.376			−4.0%
9, 10	$CaCO_3$	TMAH 1%	0.370			−5.5%
11, 12	$CaCO_3$	no treatment	0.392			
13	$CaCO_3$ + *Chlorella* 1[c]	no treatment	32.56			
14	$CaCO_3$ + *Chlorella* 1[c]	H_2O_2/NaOCl	0.983	95.8%	99.4%	159%
15	$CaCO_3$ + *Chlorella* 1[c]	Boyle	1.190	96.2%	99.1%	214%
16	$CaCO_3$ + *Chlorella* 1[c]	H_2O_2	1.251	95.1%	98.9%	230%
17	$CaCO_3$ + *Chlorella* 1[c]	TMAH 5%	2.416	90.1%	97.1%	537%
18	$CaCO_3$ + *Chlorella* 1[c]	TMAH 1%	1.468	93.9%	98.5%	287%

[a]Cleaning efficiency, calculated as the sum of noncarbonate Mg released in oxidation (or TMAH) step and subsequent rinses divided by the total noncarbonate Mg release (oxidation and rinses plus noncarbonate Mg release on dissolution).

[b]The ratio of excess Mg released during dissolution of the cleaned sample compared to the total excess (noncarbonate) Mg upon dissolution of the composite sample without cleaning (split 13). Slight differences between cleaning efficiency and total efficiency may reflect either removal of complexed or bound Mg during rinses that was not detected by flame AA or more effective buffering of noncarbonate Mg release during dissolution of cleaned samples.

[c]Composite sample made by contaminating 5 mg $CaCO_3$ splits with 200 μL of concentrated *Chlorella* algae suspended in ethanol. The *Chlorella*, a noncalcifying marine algae, had been harvested from culture, concentrated by centrifugation, lysed via sonication in seawater media, and rinsed several times in distilled water before suspension in ethanol to avoid bacterial degradation. The composite samples were then rinsed twice in distilled water and once in ethanol.

samples which differ slightly from those used in the experiments in Tables 1 and 2. Cleaned carbonates were analyzed as described above, but cleaning and postcleaning rinse solutions were not analyzed for Mg release. We also examined release of Mg with no cleaning other than rinses in distilled water.

2.2. Effect of Treatments on Carbonate Mg/Ca

[10] Examined cleaning treatments altered the Mg/Ca ratio of the calcium carbonate phase (uncontaminated) only slightly (1.5–5% decrease; see Table 2, splits 1–12, and Figure 1). The bleach/peroxide and Boyle methods appear to have the least effect on the carbonate Mg/Ca, although precise relationships are partly obscured by analytical uncertainty of atomic absorption (AA) analysis.

2.3. Efficiency of Treatments at Removing Mg in Organic Phases in Composite Samples

[11] Different oxidizing methods and the TMAH methods all significantly reduced the Mg/Ca ratios of the composite samples (from 33 to ∼1 mmol/mol (Figure 2a and Table 2, splits 14–18). However, these values are still several times higher than the Mg/Ca ratio of the carbonate fraction alone (0.4 mmol/mol), indicating incomplete removal of Mg in the organic phase. Although these cleaning treatments removed >97% of the noncarbonate Mg, the remaining noncarbonate Mg significantly alters the measured carbonate ratio. This may be exacerbated by loss of ∼40–50% of the carbonate during cleaning from dissolution (∼15%) and physical losses during siphoning/pipetting (∼30%).

Table 3. Comparison of Cleaning Procedures for $CaCO_3$ and Chlorella Using 1:1 Peroxide/Bleach

Split	Sample	Rinse NH_4OH (20 mL)[a]	Rinse DI (20 mL)[a]	Times oxidized (H_2O_2/NaOCl)[a]	Rinse NH_4OH (20 mL)[a]	Final rinses DI (5 mL ea.)[a]	Mg/Ca carb (mmol/mol)	Total Efficiency[b]	Percent Difference Carbonate Mg/Ca
A	$CaCO_3$ + *Chlorella* 2[c]	0	0	1	0	20	0.52	99.6%	31.7%
B	$CaCO_3$ + *Chlorella* 2[c]	0	0	1	1	20	0.44	99.9%	11.8%
C	$CaCO_3$ + *Chlorella* 2[c]	0	0	2	1	20	0.46	99.9%	17.4%
D	$CaCO_3$ + *Chlorella* 2[c]	0	0	2	0	20	0.48	99.8%	21.5%
E	$CaCO_3$ + *Chlorella* 2[c]	0	1	1	0	20	0.42	99.9%	7.9%
F	$CaCO_3$ + *Chlorella* 2[c]	1	0	1	0	20	0.50	99.7%	28.2%
G	$CaCO_3$ + *Chlorella* 2[c]	0	0	0	0	0	43.62		

[a]Succession of cleaning steps applied, as described in section 3.1. All rinse solutions prior to oxidation were shaken with the sample for 30 min.

[b]As calculated in Table 1.

[c]The second set of composite samples, prepared by adding 100 mg of reagent $CaCO_3$ powder to the pellet of centrifuged *Chlorella*. The mixture was sonicated, rinsed with distilled water, and stored in 25% methanol for 2 days. The mixture was then diluted with distilled water and split equally into 20 centrifuge tubes, and nearly all the liquid was removed by centrifuging. These splits were capped and stored at room temperature for 2 months prior to additional cleaning experiments, except for four splits in which we examined Mg release in rinses with distilled water. The dry weight of the *Chlorella* in each split was ~5 mg (1:1 *Chlorella*:$CaCO_3$ by weight).

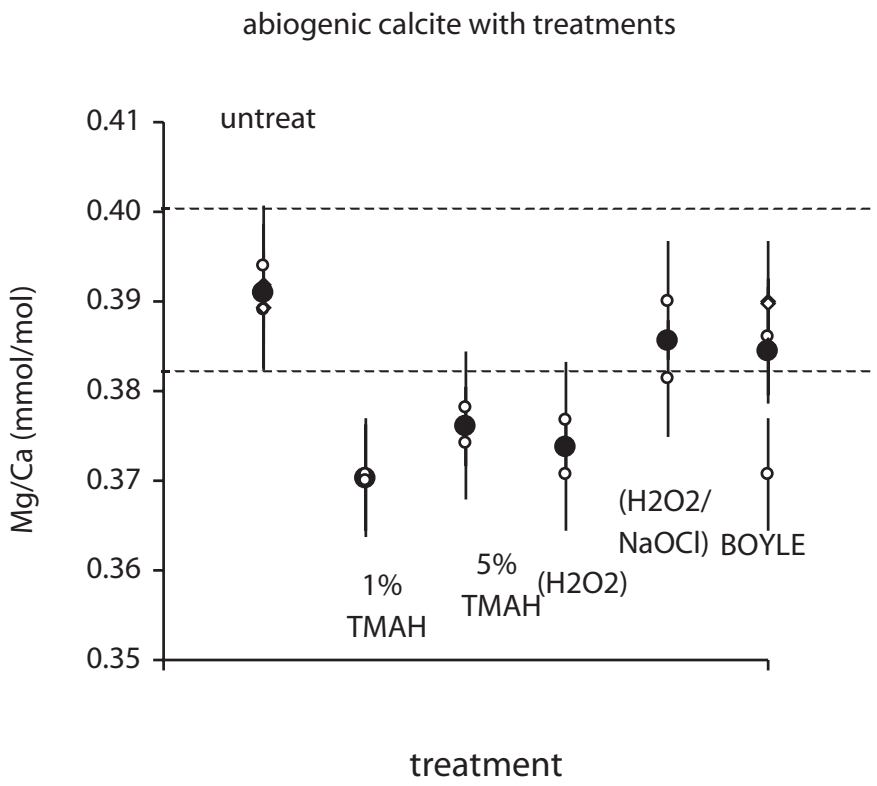

Figure 1. Effect of various treatments on Mg/Ca of reagent $CaCO_3$ (with no organic contamination, splits 1–12 in Table 2). Open symbols indicate replicates, and large solid samples represent the average. Error bars on each replicate indicate the uncertainty of AA analyses.

[12] While the total removal of organic Mg was generally similar in the different treatments, the rate of Mg removal differed greatly (Figure 2b). The most efficient cleaning methods appear to be either the bleach/peroxide method or the concentrated peroxide method, both of which released large amounts of Mg rapidly. However, neither cessation of Mg release (or plateau in Mg release) in cleaning/rinse solutions nor reproducibility of measured Mg/Ca ratios with different types of cleaning treatments are sufficient criteria to assess the complete removal of contaminating Mg in carbonate samples. Consequently, a single application of these tested methods may not be sufficient to remove organic-bound Mg from many sample types. This would depend on the amount of organic matter present in the original sample. In section 3, we propose criteria for evaluating whether cleaning techniques are sufficient to permit analysis of coccolith carbonate Mg/Ca.

[13] Further experiments with the bleach/peroxide method were much more successful at recovering carbonate Mg/Ca ratios, but this appears to result in part from natural bacterial oxidation during the long storage of the second set of composite samples (Table 3). Split A (Table 3), stored for 2 months prior to cleaning, received the same oxidation procedure as split 14 (Table 2) but had much more efficient Mg removal during cleaning. After cleaning, Mg/Ca ratios of the second set of composite samples were only 8–32% higher than the carbonate fraction. Noncarbonate Mg was not always more efficiently removed in splits with

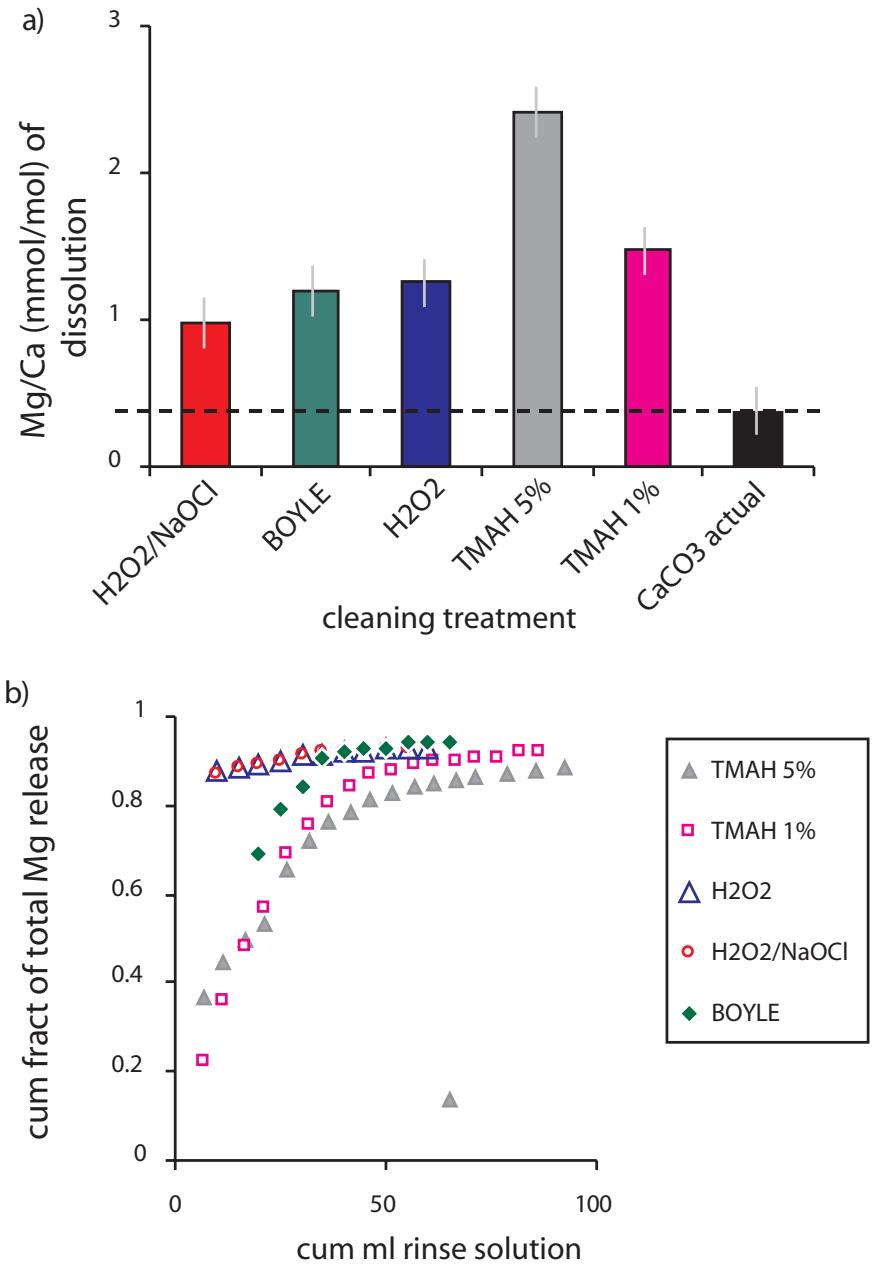

Figure 2. (a) Mg/Ca ratios of composite samples ($CaCO_3$ plus *Chlorella* 1, splits 14–18 in Table 2) after different oxidizing and tetramethlyammoniahydroxide (TMAH) treatments compared with uncontaminated $CaCO_3$. Error bars indicate the uncertainty of AA analyses. (b) Release rate of Mg from cleaning solutions and rinses following various cleaning reactions. The leftmost point for each series represents the cleaning solution, with additional distilled water added in some cases.

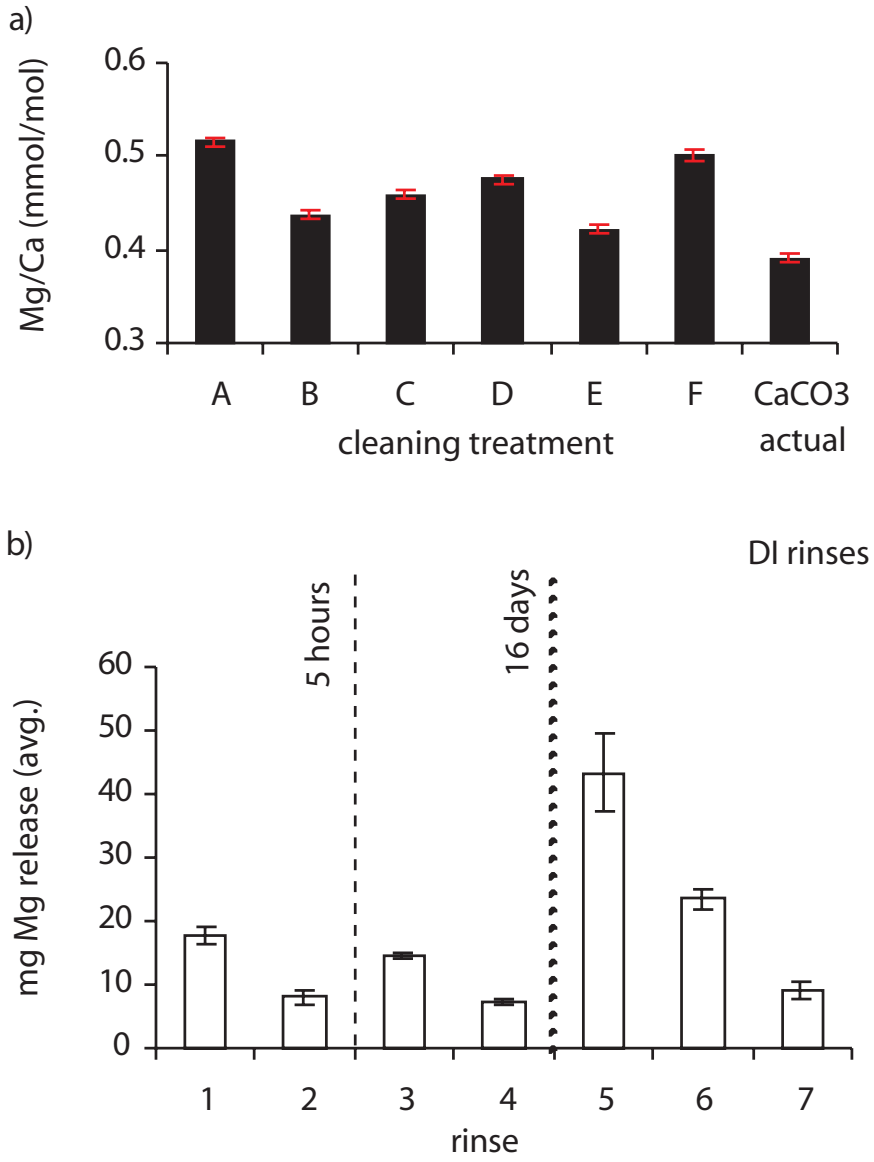

Figure 3. (a) Mg/Ca ratios of carbonate samples comparing cleaned composite samples ($CaCO_3$ plus *Chlorella* 2, splits A–F in Table 3) with uncontaminated $CaCO_3$. Methods for splits A–F are given in Table 2. Error bars indicate the uncertainty of AA analyses. (b) Average Mg release in successive distilled water rinses (prior to sample cleaning) of composite ($CaCO_3$ plus *Chlorella* 2) samples at different time intervals. For these four splits, 5 mL of distilled water (pH adjusted to 8.5 with NH_4OH) was added to each tube and then immediately centrifuged, and the liquid was removed and reserved for analysis. Rinses were initiated just after splitting composite samples. Broken lines with text boxes indicate time elapsed between successive rinses. Error bars indicate 1 standard deviation on Mg release.

two applications of the oxidizing step (splits C and D) compared with identical treatment and a single oxidation step (splits B and A, Figure 3a and Table 3). Patterns of Mg release in distilled water rinses of composite samples also suggest the importance of natural oxidation. Mg release was always highest in the first rinse after an extended period of storage, indicating that it is not the actual distilled water rinse step which releases Mg but, rather, the slower bacterially enhanced oxidation of the algal organic matter during sample storage (Figure 3b). Consequently, it may be useful to rinse poisoned or sterile culture or trap samples with distilled water, so that bacterial action in the residual water may facilitate Mg release from algal organic matter.

2.4. Application to Coccolith Samples From Culture

[14] Coccolith culture samples were cleaned following the bleach/peroxide method of section 2.1, except that prior to the oxidizing step, samples were rinsed multiple times in 2–4 mL of distilled water (brought to pH of 8.5 with NH_4OH) until Mg in rinse fluids was below detection by flame AAS. Mg release was monitored in the oxidizing solutions and subsequent rinses. Following rinses, we dissolved a small aliquot of the cleaned samples to determine the amount of carbonate present and evaluate whether cleaning procedures had eliminated sufficient Mg to permit analysis of carbonate Mg. We then selected samples that satisfied the following criteria: (1)Upon dissolution of the sample, would the noncarbonate Mg represent >10% of the Mg present? We assessed this assuming that the Mg released in rinse solutions represented 90% of the noncarbonate Mg present (on the basis of experiments in section 2.3) and assuming coccolith carbonate Mg/Ca ~0.2 mmol/mol (in culture samples with nearly no release of Mg during oxidative cleaning.) (2) Is there sufficient carbonate for analysis (>75 μg $CaCO_3$ to produce 500 μL of solution at >60 ppm Ca)?

[15] Of 42 culture samples cleaned with this method, only 11 satisfied both criteria. We attempted a second oxidation treatment on some samples excluded on the basis of the first criterion, but following reoxidation and rinses, these samples had insufficient carbonate for analysis.

[16] The 11 admitted samples were transferred to clean acid-washed tubes for dissolution in the same batch of 50 μL of ultrapure 2% HNO_3 and diluted to 500 μL with the same batch of Milli-Q distilled water and adjusted to concentrations of 65–100 ppm Ca (volume of 450 μL).

3. Methods for ICP-MS Analysis of Mg/Ca in Small Coccolith Samples

[17] Precise measurement of coccolith Mg/Ca ratios is much more challenging than measurement of foraminiferal Mg/Ca because of the small size of typical coccolith culture samples ($\ll$1 mg of carbonate) and the very low Mg content of coccolith carbonate. Coccolith Mg/Ca analysis is more complicated than coccolith Sr/Ca analysis because of the lower abundance of Mg in coccolith calcite and more pervasive contamination of Mg in the sample introduction system. We describe a method for precise analysis of coccolith Mg/Ca in microsamples using sector field ICP-MS, Finnegan model ELEMENT. Analytical settings were selected to maximize sensitivity of Mg detection and minimize the sample volume required for analysis and are summarized in Table 4.

[18] Routine techniques for cleaning the sample introduction system (e.g., soaking in or aspirating nitric acid) are not sufficient to eliminate some types of Mg contamination at the level required for these analyzes. Soaking the nebu-

Table 4. Analytical Conditions for ICP-MS measurements

Parameter	Setting	Rationale
Detection mode	Pulse counting	higher sensitivity
Resolution, m/Dm	300 (low resolution)	higher sensitivity (cannot use medium resolution to resolve interferences due to low Mg concentration)
Mass	^{24}Mg	most abundant isotope (higher counts yield better precision) but potential interferences from C_2^+, NaH^+, and $^{48}Ca^{++}$)
Mass	^{25}Mg	lower counts but fewer interferences than ^{24}Mg
Mass	^{43}Ca	low isotopic abundance (0.135%) permits detection in pulse-counting mode despite high sample Ca/Mg
Nebulizer	Teflon microconcentric	high sensitivity at low flow rates
Sample aspiration rate (peristaltic pump)	100mL/min	low flow rate given small sample volume
Spray chamber	Cyclonic	minimize rinse time and memory between samples (alternations of blanks and samples confirms negligible memory)
Sensitivity ^{43}Ca	1,400,000 cps per 100 ppm	optimized
Sensitivity ^{24}Mg	65,000 cps per 11 ppb	optimized
Scan type	E-scan	
Mass window	5%	
Sample time	0.00405 sec.	
Samples per peak	200	
Settling time		
^{24}Mg	0.05 sec	
^{25}Mg	0.001 sec	
^{43}Ca	0.055 sec	
Scans (passes times runs)	520 (=130 × 4)	
Carrier gas flow	1.3 L/min	
Auxiliary gas flow	1 L/min	
Cool gas flow	15 L/min	
Total analysis time	3 min, 30 s	

lizer, spray chamber, and torch canal in an oxidizing solution of 8% H_2O_2 and 0.8% NaOCl in distilled water (pH∼8) for 30 min with ultrasonication reduced Mg blanks from 500 ppb (after nitric acid wash) to <1 ppb Mg. Mg in typical coccolith culture samples may be only 10–20 times the acid blank, so blank subtraction is essential for precise analysis. Blanks and standards were remeasured after every three sample analyses.

[19] For the Mg/Ca ratios of these samples, sample dilution to 65–100 ppm Ca maximizes the signal/blank ratio for Mg without introducing significant artifacts in the Mg/Ca ratio due to detector dead time at high counts of ^{43}Ca. Mass bias and drift on the measured 24/43 and 25/43 ratios are calculated from a matrix-matched gravimetric standard of Mg/Ca of 0.17 mmol/mol as in the work by *Rosenthal et al.* [2000]. Detector dead time (measured in dilutions of the same matrix-matched gravimetric standard) produces a linear increase in measured Mg/Ca as Ca concentration increased to 1.5 million counts per second (Mcps) on ^{43}Ca. With increasingly concentrated solutions

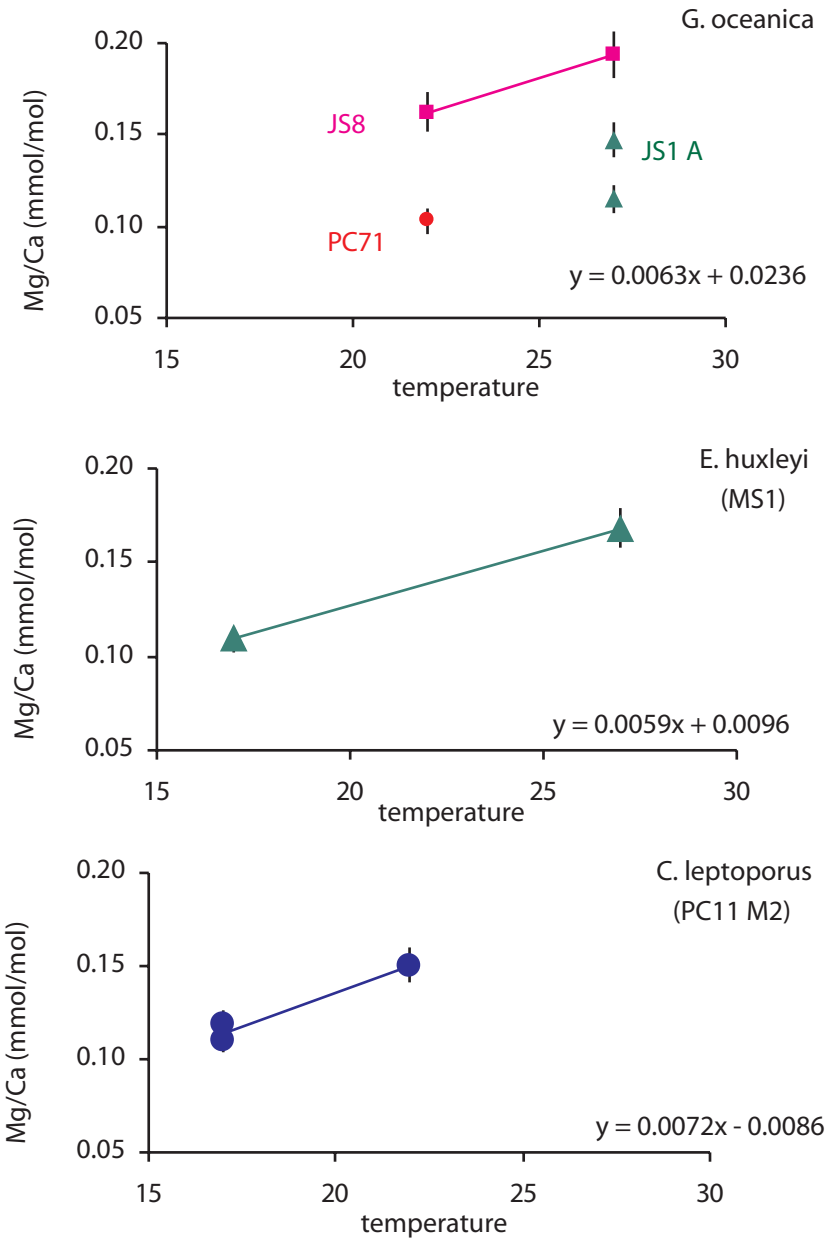

Figure 4. Mg/Ca ratios in different species of coccoliths from culture after cleaning with bleach/peroxide method. The strain cultured is given in parentheses for *Calcidiscus leptoporus* and *Emiliania huxleyi* and in the plot for *Gephyrocapsa oceanica*. Error bars shown are ±5% estimated uncertainty from possibly incomplete removal of organic phases. Analytical uncertainty is estimated at <1% relative standard deviation.

and higher cps, Mg/Ca ratios decrease, presumably because of space-charge effects on Mg detection. This does not affect measurements of samples since the intensity of counts on ^{43}Ca was <1.5 Mcps for all analyzed samples.

[20] While 24/25 ratios in the blank matched natural abundance ratios (8.9), the standards and samples had elevated 24/25 ratios (9.0), probably from $^{48}Ca^{++}$ interference due to high Ca concentrations in solutions. However, this should not affect the accuracy of the Mg/Ca determination since samples and standards contained similar Ca concentrations. Furthermore, the correlation of 24/43 and 25/43 ratios was 0.99, indicating that sample variations reflect varying Mg/Ca ratios and not variable interferences on the 24/43 ratio. The 24/43 ratio is used to calculate Mg/Ca because its higher counts yield better precision (average relative standard deviation (RSD) for four runs of each sample was 0.65%).

4. Mg/Ca in Coccoliths From Culture

[21] Measured Mg/Ca ratios of the coccolith samples range from 0.1 to 0.2 mmol/mol, 1–2 orders of magnitude lower than in foraminiferal calcite. For *E. huxleyi* and *C. leptoporus*, Mg/Ca is higher in the sample cultured at higher temperature (Figure 4). In *G. oceanica* the larger number of samples exposes greater variability in Mg/Ca even in samples from the same culture taken on different days (JS1A at 27°C). Either factors other than culture temperature may influence Mg partitioning in coccolith calcite, or noncarbonate Mg biases the higher measurement. While complications with cleaning issues and the small number of samples preclude confirmation of a temperature effect on Mg in coccolith calcite, the data are suggestive. Interestingly, the trends of Mg/Ca with temperature show similar slopes of ~6% increase in Mg/Ca per degree Celsius temperature increase.

5. Conclusions

[22] Experiments with synthetic samples of carbonate and algal organic matter suggest that the most effective cleaning treatment, causing least effect on carbonate chemistry and most complete and rapid oxidation of algal organic matter, is oxidation in an equal volume mixture of sodium hypochlorite (2.8%) and hydrogen peroxide (30%). However, owing to the large contrast in Mg contents of organic and carbonate fractions, persistence of even a small fraction of the organic matter (<1%) can alter measured carbonate Mg/Ca ratios. In some culture or sediment trap samples with very high organic matter contents this method may not remove enough organic Mg to permit precise measurement of coccolith Mg/Ca.

[23] The combination of small sample size and low Mg/Ca ratios requires very low analytical blank levels, which are best obtained by cleaning the entire sample delivery system with the same oxidizing solutions used to clean samples (diluted 50% with distilled water). It is possible to attain very precise measurements of sample Mg/Ca (<1% RSD) in pulse-counting mode on a sector field ICP-MS (Finnigan model ELEMENT) using matrix-matched standards to correct for drift and detector dead time. Ultimately, it is likely to be the efficiency of sample cleaning methods that determine the precision and utility of measurements of coccolith Mg/Ca.

[24] Measurements of Mg/Ca in coccoliths from several species grown in culture suggest that temperature may be an important control on Mg partitioning in coccolith calcite, but further studies are needed to confirm this result. Because coccoliths are much smaller and have a much lower Mg content (compared to foraminifera), the potential advantages of a coccolith Mg/Ca paleotemperature proxy may be outweighed by the greater complexity of cleaning issues.

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