Growth rate dependence of Sr incorporation during calcification of *Emiliania huxleyi*

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[1] Reconstructing the dissolved carbon dioxide of surface waters from the $\delta^{13}C$ of organic carbon relies on accurate determination of the growth rate of phytoplankton contributing to sedimentary organic matter. We demonstrate that the Sr/Ca ratio in the coccoliths of *Emiliania huxleyi* is correlated with the rates of both organic carbon fixation and calcification. An investigation of biomineralization models suggests that these three factors may be linked by the differential pumping rates of calcium relative to strontium ions across the cellular membranes which supply ions for photosynthetic carbon fixation and calcification and derives energy from photosynthetic products. A quantitative relationship is derived between calcification, organic carbon fixation, and Sr/Ca such that a combined geochemical proxy approach using ε_p and Sr/Ca can be applied to the paleoceanographic record. *INDEX TERMS:* 1045 Geochemistry: Low-temperature geochemistry; 4808 Oceanography: Biological and Chemical: Chemical tracers; 4855 Oceanography: Biological and Chemical: Plankton; 4875 Oceanography: Biological and Chemical: Trace elements; *KEYWORDS:* Sr/Ca, growth rate, *E. huxleyi*, coccolithophore, proxy

1. Introduction

[2] Bubbles of past atmosphere trapped in polar ice caps suggest an intricate relationship between atmospheric carbon dioxide (pCO_2) and climate change [*Petit et al.*, 1999]. One approach to understanding the natural fluctuations in pCO_2 is to chart the evolution of critical areas of the surface ocean as a source or sink of CO_2 [e.g. *Rosenthal et al.*, 2000]. This approach requires a direct proxy for the concentration of carbon dioxide dissolved in surface waters ($[CO_2(aq)]$). One such proxy is the isotopic fractionation of carbon between seawater and organic matter [*Jasper and Hayes*, 1990; *Jasper et al.*, 1994]. The faster assimilation of ^{12}C relative to ^{13}C by the enzyme Rubisco leads to a kinetic fractionation between the carbon isotopes during carbon fixation. The difference in $\delta^{13}C$ between dissolved inorganic carbon and organic matter (ε_p) depends on the rate of supply of carbon relative to the rate of photosynthetic carbon fixation by the cell [*Farquhar et al.*, 1982; *Goericke et al.*, 1994]. Carbon may be supplied to the cell by passive diffusion such that the rate of supply is proportional to the $[CO_2(aq)]$. Recent evidence suggests that marine phytoplankton also have the capacity for active C transport and/or catalyzed conversion of HCO_3^- to CO_2 by carbonic anhydrase [*Tortell et al.*, 1997, 2000]. When the rate of carbon supply is infinite relative to the rate of carbon fixation, then maximal fractionation (~25‰) is observed. Alternatively, when the rate of carbon fixation approaches the rate of carbon supply, the isotopic composition of the organic matter tends toward that of the carbon source, and no fractionation is observed. Recent continuous culture experiments have exploited this relationship between ε_p and $[CO_2(aq)]$ to calculate the growth rate of phytoplankton in the modern ocean [*Laws et al.*, 1995]. For paleoceanographic applications, workers have chosen to analyze cores from oligotrophic areas where it may be reasonable to assume a constant growth rate in order to calculate pCO_2 [e.g., *Bidigare et al.*, 1997; *Pagani et al.*, 1999; *Popp et al.*, 1999; *Laws et al.*, 2001]. In order to reconstruct surface water carbon dioxide concentrations more accurately, ε_p must be corrected by an independent measure of the carbon specific growth rate [e.g., *Francois et al.*, 1993; *Bidigare et al.*, 1997; *Laws et al.*, 2001].

[3] In some of the original studies using ε_p to reconstruct pCO_2, $\delta^{13}C$ values were measured on alkenones because they are produced only by haptophyte algae and are thought to be resistant to diagenetic alteration. An important advantage of using ε_p calculated from isotopic analyses of alkenones is that haptophytes have a limited size range and so the geometry of the alkenone-producing cell can be constrained [e.g., *Popp et al.*, 1998; *Burkhardt et al.*, 1999; *Riebesell et al.*, 2000b], which is an additional theoretical control on ε_p [e.g., *Francois et al.*, 1993; *Goericke et al.*, 1994; *Rau et al.*, 1996]. *Bidigare et al.* [1997] emphasized the importance of an independent proxy for growth rate for CO_2 paleobarometry. They suggested that owing to the close relationship between phosphate concentrations and growth rate, planktonic foraminiferal Cd/Ca may be applied to sediment cores to obtain phosphate concentrations and therefore growth rate. However, this approach is limited to areas of nonzero phosphate concentrations, and the application of planktonic foraminiferal Cd/Ca as a proxy for phosphate is not straightforward [*Rickaby and Elderfield*, 1999]. More recently, *Bidigare et al.* [1999] proposed that the $\delta^{13}C$ of phytol, an algal biomarker, may record phytoplankton growth rate. In this study we propose an alternative proxy for growth rate which is also specific to the producers of the alkenones, mainly the coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Previous studies have demonstrated that calcification rates of these organisms are related to particulate organic carbon (POC) produc-

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Table 1. Target Nutrient and Salt Concentrations in the Growth Medium

	Tank 1	Tank 2	Tank 3
Nutrients			
DIN, μM	2.0	2.0	2.0
PO_4^{2-}, μM	36.25	36.25	36.25
Vitamins	f/2	f/2	f/2
Metals	f/2	f/2	f/2
DIC, μM	2200	2200	2200
Salts			
NaCl, M	0.4	0.4	0.4
$MgCl_2$, mM	24	24	24
$MgSO_4$, mM	24	24	24
$CaCl_2$, mM	7	7	7
KCl, mM	7	7	7
Salinity	35	32	31

tion [*Balch and Kilpatrick*, 1996]. This suggests that the coccoliths, the calcite platelets produced by the coccolithophorid, may record in their chemistry some measure of coccolithophore growth rate. Indeed, previous studies of the chemistry of coccoliths show a positive correlation between carbon and oxygen isotopes [*Dudley and Nelson*, 1994], consistent with a kinetic control similar to what has been proposed for scleractinian corals [*McConnaughey*, 1989]. This kinetic effect, associated with the precipitation rate, should also be reflected in the trace element composition of the coccoliths. It should be noticed that the absolute ratio of calcification to POC production can change according to factors such as availability of CO_2(aq) [*Riebesell et al.*, 2000a] and nutrients [*Van Bleijswijk et al.*, 1994; *Paasche and Brubak*, 1994].

[4] In inorganic systems, calcite precipitation experiments have shown a positive correlation between the partition coefficient of Sr into calcite D_{Sr} and the rate of precipitation [*Lorens*, 1981; *Tesoriero and Pankow*, 1996]. This change in calcite chemistry has been attributed to discrimination against incorporation of the larger, disruptive Sr^{2+} into the calcite lattice (Sr^{2+} = 0.113 nm; Ca^{2+} = 0.099 nm) at low precipitation rates, which becomes less selective as precipitation rates increase. The Sr/Ca of the calcite ultimately tends toward the composition of seawater at very high precipitation rates. The kinetics of surface reactions have also been proposed to account for the correlation [*Morse and Bender*, 1990; *Watson*, 1996].

[5] *Stoll and Schrag* [2000] investigated whether observations from inorganic systems could be applied to the chemistry of the biogenic calcite of coccolithophorids and hence yield a sedimentary proxy for phytoplankton growth rate. They observed variations in Sr/Ca of coccolithophores from core tops and down core records from the equatorial Pacific of up to 20%. According to their earlier modeling studies [*Stoll and Schrag*, 1998], it is impossible to explain these modern-day and glacial-interglacial variations by changes in the Sr/Ca of the surface ocean. They proposed that differences in coccolith Sr/Ca were due to variations in growth or calcification rate. However, isolation of the specific effect of growth rate on the chemistry of the coccolith calcite in the modern ocean remains a challenge.

[6] In this study we take advantage of batch culture experiments where it is possible to carefully control some aspects of the growth conditions. Mesocosm culture experiments with *Emilania huxleyi* were performed to confirm the relationship between phytoplankton growth rate and D_{Sr}. We present a relationship between the D_{Sr} of the calcite coccoliths and the ε_p measured in the organic matter which has the potential to be used to reconstruct surface water CO_2(aq). We discuss these results in the context of other culture results (H. M. Stoll et al., Calcification and temperature effects on Sr partitioning in coccoliths of multiple species of coccolithophorids in culture, submitted to *Global Planetary Change,* 2001) (hereinafter referred to as Stoll et al., submitted manuscript, 2001) and with respect to theories for minor element incorporation during biomineralization. It should be noted that unlike continuous cultures, a batch culture is analogous to a coccolithophorid bloom situation where cells grow exponentially in a nutrient-replete medium until growth is limited by nutrient availability. A thorough investigation of secondary effects, such as light intensity and the growth limiting resource, which are thought to be additional factors influencing ε_p [*Laws et al.*, 1995; *Burkhardt et al.*, 1999; *Riebesell et al.*, 2000a, 2000b], was beyond the scope of this study.

2. Experimental Method

2.1. Materials

[7] *E. huxleyi* strain PMLB92/011 was grown in three 1 m^3 polyethylene tanks at different CO_2 concentrations under controlled conditions of light, temperature, and nutrients. The growth medium was artificial seawater made of deionized water with added nutrients and salts as listed in Table 1. In order to force the cultures into nutrient limitation at cell densities typically observed in the natural environment the cultures were started at low concentrations of dissolved inorganic nitrogen (DIN). Concentrations of phosphate, metals, and vitamins were added according to f/2 [*Guillard and Ryther*, 1962]. Dissolution of the salts (especially NaCl) turned out to be somewhat problematic, causing slight differences in salinity between the three tanks. Also, initial DIN concentrations differed, with concentrations of 0.27, 1.8, and 1.9 $\mu mol\ L^{-1}$ in tanks 1, 2, and 3, respectively. Before filling the experimental tanks, the medium was filtrated over a 0.2 μm polyacetate filter. Initial concentration of dissolved inorganic carbon (DIC, added from a 1.42 $mol\ L^{-1}$ Na_2CO_3 stock solution) was 2255 $\mu mol\ L^{-1}$. In order to manipulate the CO_2 concentration the pH was adjusted by addition of HCl or NaOH, yielding initial pCO_2 values of 377, 582, and 960 μatm and alkalinities of 2595, 2493, and 2411 $\mu mol\ L^{-1}$ in tanks 1, 2, and 3, respectively. The mesocosms were inoculated with *E. huxleyi* from precultures, where cells had been grown under the experimental conditions for at least seven cell divisions prior to the start of the experiment. Free gas exchange between the experimental medium and the atmosphere was allowed, causing a continuous drift in pCO_2 values toward atmospheric equilibrium in each of the tanks. Temperature, light intensity, and pH were measured on line with a licor data logger. The tanks were illuminated from one side, resulting in a light intensity of 125 $\mu mol\ s^{-1}\ m^{-2}$ at the exposed side, 95 $\mu mol\ s^{-1}\ m^{-2}$ in the middle of the tanks, and 65 $\mu mol\ s^{-1}\ m^{-2}$ at the dark side. Light-dark cycle was 16:8 hours. Mean temperature over the whole experiment was 14.6°C, with minimum and maximum temperatures of 13.9° and 16.1°, respectively. The experiment was run over 20 days from 3 to 23 September 1999. From day 15 to the end of the experiment the mesocosms were kept in the dark. The tanks were mixed shortly before sampling once a day, 1–2 hours before the start of the dark phase.

2.2. Carbon System and Isotope Analysis

[8] Subsamples for DIC, δ^{13}C-DIC, and alkalinity determination were filtered through a 0.45 μm cellulose acetate filter. Samples for DIC and alkalinity were stored in 300 mL borosilicate bottles and samples for δ^{13}C-DIC were stored in 30 mL borosilicate bottles, fixed with 1 and 0.01 mL $HgCl_2$ solution (35 $g\ L^{-1}$), respectively, and refrigerated at 4°C. DIC was measured coulometrically [*Johnson et al.*, 1985] in duplicate, and alkalinity was calculated from linear Gran plots [*Gran*, 1952] after duplicate potentiometric titration [*Bradshaw et al.*, 1981; *Brewer et al.*, 1986]. The CO_2 concentration in the medium was calculated from temperature, salinity, and the concentrations of DIC, alkalinity, and PO_4, using the equilibrium constants of *Goyet and Poisson* [1989]. Extractions

and measurements for the determination of δ^{13}C-DIC were performed in the laboratory of H. J. Spero, University of California Davis, with a precision of ±0.11‰.

[9] Subsamples for the determination of particulate organic nitrogen (PON), total particulate carbon (TPC), and particulate organic carbon were filtered on precombusted (12 hours, 500°C) GF/F filters (Whatman) and stored at −20°C. Before measuring, the POC filters were fumed for 2 hours with a saturated HCl solution to remove all inorganic C. PON, TPC, and POC were subsequently measured on an ANCA-SL 20-20 Europa Scientific mass spectrometer. Stable carbon isotopes of TPC and POC were measured simultaneously. Particulate inorganic carbon (PIC) was calculated as the difference between TPC and POC. Carbon content of bacterial biomass as calculated from cell counts and size measurements was subtracted from POC and TPC for correction. The isotopic composition of CO_2 (δ^{13}C-CO_2) was calculated from δ^{13}C-DIC using the equation by *Rau et al.* [1996] based on *Mook et al.* [1974]:

$$\delta^{13}\text{C-CO}_2 = \delta^{13}\text{C-DIC} + 23.644 - \left(\frac{9701.5}{T_k}\right). \quad (1)$$

Carbon isotope fractionation during the synthesis of POC (ε_P) was calculated relative to the isotopic composition of CO_2 in the medium according to *Freeman and Hayes* [1992]:

$$\varepsilon_{\text{POC}} = \frac{\delta^{13}\text{C-CO}_2 - \delta^{13}\text{C-POC}}{1 + \frac{\delta^{13}\text{C-POC}}{1000}}, \quad (2)$$

where δ^{13}C-POC is the isotopic composition of POC.

2.3. Sr/Ca Analysis

[10] The coccolithophore samples were captured on precombusted (12 hours, 500°C) GF/F (Whatman) filters. One sample comprised a quarter of a filter, which was placed in an acid-washed 1.5 mL microcentrifuge tube. As these were culture samples, no cleaning was required for potential sediment contamination. However, owing to the large quantity of organic matter, an oxidative cleaning step involving boiling in 1 mL of NaOCl for 2 hours was employed in order to avoid problems with the plasma during analysis. After thorough rinsing with ethanol, the samples were treated with 0.5 mL of 10% HNO_3 to ensure complete dissolution of all the carbonate coccoliths present. A small aliquot of the resulting solution was diluted to 2 mL and analyzed so that the amount of calcium present in the original solution could be calculated. Appropriate dilutions were then made to achieve constant concentrations of all the samples and avoid the small effects of Ca on the Sr/Ca measured ratio [*Schrag*, 1999]. For analysis of the medium an aliquot of the medium was removed and diluted with 2% HNO_3 to avoid any matrix effects between the standards and the samples. Sr/Ca ratios were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on a Jobin-Yvon simultaneous instrument, model 46-P. The resulting precision is better than 0.5% on the basis of replicate analyses of the same sample at different dilutions over the course of several days. The partition coefficient of Sr between the medium and the coccoliths is calculated according to

$$D_{\text{Sr}} = \frac{\left(\text{Sr}/\text{Ca}\right)_{\text{coccolith}}}{\left(\text{Sr}/\text{Ca}\right)_{\text{medium}}}. \quad (3)$$

2.4. Growth Rate Analysis

[11] Growth rate μ during the culture experiment was estimated from the increase in number of cells, where subsamples for cell counts were fixed with 400 μL 20 mL^{-1} sample of a 20% hexamine-buffered formaldehyde solution and stored at 4°C. Cell abundance was determined by means of a Coulter Multisizer II particle counter. At least 5700 cells per sample were counted. Cell-specific growth rate was calculated according to

$$\mu = \frac{\ln N_{\text{fin}} - \ln N_o}{\Delta t}, \quad (4)$$

where N_o and N_{fin} are the cell concentrations at the beginning and the end of the experiment and Δt is the duration of the experiment in days. In order to correct for the effects of day length and respiration on the measured growth rate the instantaneous growth rate μ_i was calculated as

$$\mu_i = \frac{(L + D)[\ln(N_i) - \ln(N_o)]/t}{(L - Nr)}, \quad (5)$$

where N_0 is the cell concentration at the beginning of the experiment day x, N_1 is the cell concentration at the end of the experiment day $x + 1$, t is the duration of the incubation in days, L and D are the duration of the light and dark period, respectively, in hours, and r is the rate of carbon loss due to respiration in the dark and is assumed to equal 15% of the photosynthetic carbon assimilation rate during the preceding light period [*Laws and Bannister*, 1980; *Laws et al.*, 1995]. Growth rates for particulate organic carbon μ_{POC} and particulate inorganic carbon μ_{PIC} were calculated by substituting in (4) initial and final POC and PIC concentrations, respectively, e.g., substituting POC_0 and POC_i, respectively. It should be noted that μ_{PIC} and μ_{POC} are the best experimental estimates of the growth rates in terms of particulate inorganic and organic carbon. But neither of these measurements is strictly equivalent to the carbon-specific growth rate μ_C, which controls the isotopic fractionation during photosynthetic carbon metabolism. The results from tank 1 will not be discussed here because the coccolithophores in this tank may have suffered salinity shock.

3. Results

[12] After a lag phase of ~3 days, cells grow exponentially from day 4 to day 8, with the highest growth rate (1.21 and 1.17 $days^{-1}$ in tanks 2 and 3, respectively) reached on day 6 (Figure 1a). After the exhaustion of DIN on day 6, the cells divide about one more time before going into the stationary phase. This increase in the concentration of coccolithophores is insufficient to affect the Sr/Ca of the large volume of artificial seawater. The absolute Sr/Ca ratio (9.7 ± 0.2 mmol mol^{-1} on 30 samples) of the medium, however, is slightly higher than that of average modern-day seawater (8.5 mmol mol^{-1}). The data from this culture experiment will be presented and discussed in terms of the partition coefficient of Sr between the medium and the coccoliths ($D_{\text{Sr}} = (\text{Sr/Ca})_{\text{coccolith}}/(\text{Sr/Ca})_{\text{medium}}$) so that the culture data may be compared directly and applied to data from sediment cores. This measured D_{Sr} is a cumulative measurement and reflects an integration of the Sr/Ca of all the coccolithophorids which have grown until that sampling day. In order to compare how the Sr partitioning is affected by instantaneous growth rate we also calculate the incremental Sr/Ca, which has been added since the previous sampling. It should be noted that oceanic sediments also represent an integration of flux from the surface ocean and so may be better approximated by the measured cumulative D_{Sr} as opposed to the calculated incremental D_{Sr}.

[13] In each experiment the cumulative and incremental D_{Sr} of the coccoliths paralleled the change in number of cells and record high values during the exponential phase of cell division (Figure 1b). As growth rate slowed toward the stationary phase, the incremental D_{Sr}

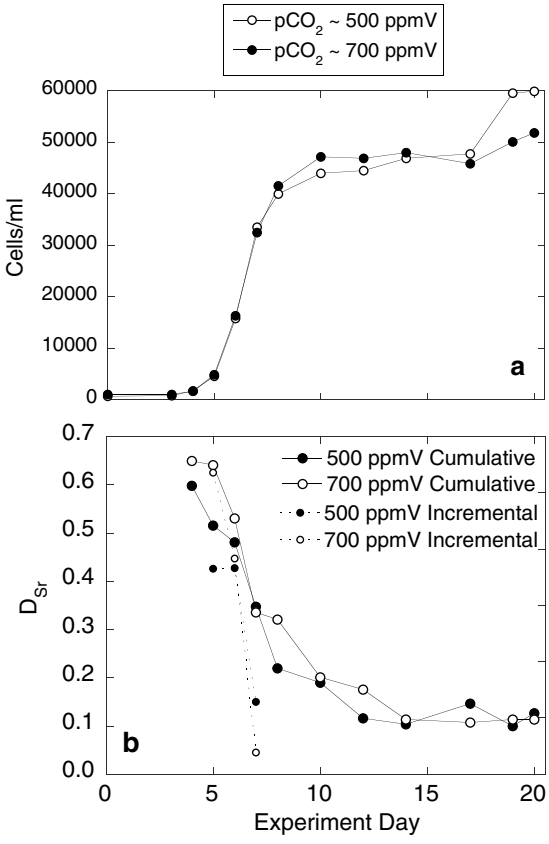

Figure 1. (a) Cell density and (b) the cumulative (solid lines) and incremental (dashed lines) partition coefficient of Sr into the coccoliths (D_{Sr}), plotted versus time (in days) in the two mesocosms with initial $pCO_2 \sim 500$ ppmv (open circles) and $pCO2 \sim 700$ ppmv (solid circles). The incremental D_{Sr} is not plotted during the stationary phase because growth rates are essentially zero and so therefore is the incremental D_{Sr}. The cumulative data do not incur this problem as the asymptote represents an integral of all coccoliths grown throughout the experiment.

decreases faster than the cumulative D_{Sr}. At high cell division rates the partition coefficient for Sr into calcite can be up to 6 times higher than during the stationary stable phase when the cumulative and incremental D_{Sr} tend toward a single value of 0.1 and 0, respectively. As may be expected, the data from the cumulative D_{Sr} acts a smoothing function to the incremental D_{Sr}.

[14] There is a good correlation between the cumulative and incremental D_{Sr} of coccoliths with all the experimental estimates of growth rate ($r^2 > 0.7$), and the best correlation exists with μ_{PIC} and cumulative D_{Sr} ($r^2 = 0.90$) (Figure 2a). The slightly greater scatter in the correlation between D_{Sr} and μ_{POC} (Figure 2b) may be caused by a decoupling of μ_{PIC} and μ_{POC} during the nutrient-limited stable phase of the experiment, as has been observed in previous studies at the onset of nutrient limitation [*Van Bleijswijk et al.*, 1994; *Paasche and Brubak*, 1994]. However, our data show equally strong correlations between D_{Sr} and μ_{POC} for exponential and stationary phases of growth, suggesting that this effect is not dominant in our experiments.

[15] If the D_{Sr} of coccoliths is going to be useful as a correction for the growth rate effect on ε_p, it must be related to μ_C, the carbon-specific growth rate, which is not measured exactly by any of our experimental estimates of growth rate. A test of cumulative and incremental D_{Sr} as a proxy for μ_C is to normalize the values to $[CO_2(aq)]$ and compare these results with cumulative and incremental ε_p. A discussion of the difference between our ε_p values from this batch culture incubation and those obtained under chemostat conditions is given by *Laws et al.* [2001]. These $[CO_2(aq)]$-normalized values for D_{Sr} show a strong linear relationship with ε_p for both the incremental and cumulative data sets (Figure 3), which suggests that D_{Sr} is a good proxy for carbon-specific growth rate μ_C. More importantly, it is evident that a combination of analyses of the Sr/Ca of coccoliths and the $\delta^{13}C$ of alkenones has the potential to be used to reconstruct past variations in surface water $[CO_2(aq)]$.

4. Discussion

[16] The range in coccolith D_{Sr} values obtained in this culture experiment is approximately an order of magnitude greater than published data of coccolith Sr/Ca ratios from box core tops and down core records from the equatorial upwelling zone of the Pacific Ocean [*Stoll and Schrag*, 2000]. Our high culture values

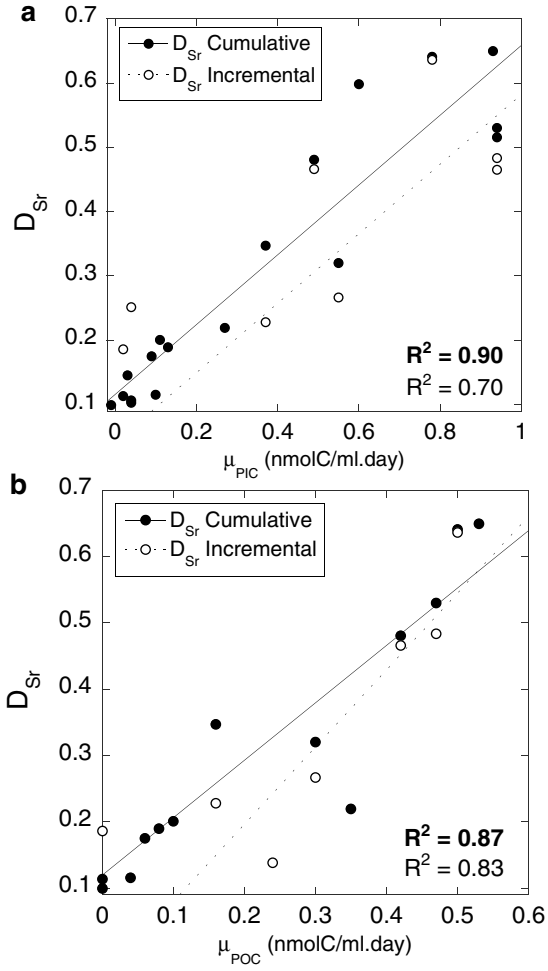

Figure 2. The cumulative (solid circles and solid line) and incremental (open circles and dashed line) strontium partition coefficient (D_{Sr}) plotted against (a) μ_{PIC} (cell-specific growth rate of particulate inorganic carbon) and (b) μ_{POC} (cell-specific growth rate of particulate organic carbon). A linear best fit yields, for μ_{PIC}, an $r^2 = 0.90$ and 0.70 for cumulative and incremental and, for μ_{POC}, $r^2 = 0.87$ and 0.83 for cumulative and incremental, respectively.

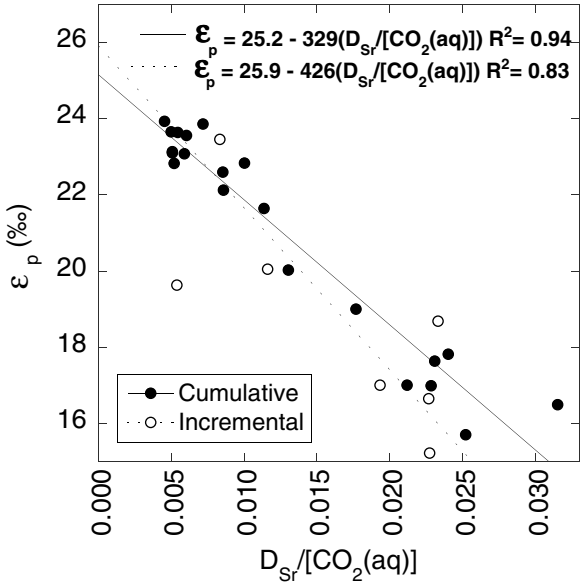

Figure 3. D_{Sr} (partition coefficient of Sr) normalized to [CO_2(aq)] and plotted against ε_p to investigate how well D_{Sr} acts as a proxy for μ_C, the carbon-specific growth rate. Cumulative values of D_{Sr} and ε_p are plotted in solid circles and with a solid line, and incremental values of D_{Sr} and ε_p are plotted with open circles and a dashed line.

(D_{Sr} = 0.6), which have not yet been observed in the ocean, may be explained by the optimal growth conditions during the early phase of the mesocosm bloom with stable light levels and very abundant nutrient concentrations. These conditions induce greater initial rates of growth and higher Sr/Ca values than found in the open ocean. The unusually low culture values (D_{Sr} = 0.1), on the other hand, may not be sampled in the sediment record as the chemical signature of the sediment coccoliths may be biased by the mixture of signals that are obtained from the different growth phases in coccolithophorid bloom areas. Alternatively, *Broerse* [2000] suggests that *E. huxleyi*'s contribution to the fine fraction of the sediments is minor owing to its small size, so the range in chemical signatures may be diluted by other more bulky coccolithophorid species. It is clear that further investigation of multiple species in culture experiments and coccolithophores from traps and ocean sediments is required to understand this system fully.

[17] D_{Sr} values measured on a variety of coccolithophorid species from culture experiments (Stoll et al., submitted manuscript, 2001) are also enveloped by the range obtained in this study, although only limited data has been published from *E. huxleyi*. Why should the coccoliths of *E. huxleyi* from this study have a more dynamic range in their Sr incorporation than the other species of coccolithophorids investigated by Stoll et al. (submitted manuscript, 2001)? While it is possible that the discrepancy may be due to differences in culturing methods, another explanation involves the potentially faster metabolism of the small *E. huxleyi* relative to other species. Another possibility is that the vertical versus radial crystallographic growth axes control the chemistry [*Young et al.*, 1992]. In particular, *E. huxleyi* is very different to most other common species in that the entire coccolith appears to be formed by a single cycle of crystal units with approximately radial *c* axes, although smaller vertically oriented crystals are grown on the template. Further investigation is necessary to confirm either hypothesis.

[18] Stoll et al. (submitted manuscript, 2001) suggest that temperature may be a significant factor in controlling the partitioning of Sr into coccolithophorid calcite. We were unable to test this hypothesis as all of our culture experiments were performed at fairly constant temperatures. Because temperature is so tightly correlated with growth rate in the experiments of Stoll et al. (submitted manuscript, 2001), it is difficult to determine whether there is an independent effect of temperature on Sr/Ca incorporation. Even if there is some temperature dependence, it may not be a significant complication in using coccolith Sr/Ca as a proxy for growth rate if there is a direct effect of temperature on growth rate.

[19] This culture experiment demonstrates the potential for the application of coccolith Sr/Ca to reconstruct growth rates from oceanic sediments and to be used in concert with ε_p from alkenones as a paleobarometer for CO_2. Coccolith Sr/Ca has the advantage of being produced by the same organism as the alkenones and recording the exact same conditions as ε_p. Moreover, the dominant control on the chemistry of coccoliths is the rate-dependent partitioning of Sr as the Sr/Ca ratio of the modern ocean varies by only 2–3% [*De Villieurs*, 1999] and can only have varied by 1–3% on timescales of the glacial-interglacial cycle [*Stoll and Schrag*, 1998]. Our approach may not be valid on longer timescales (>5 Myr) when the Sr/Ca ratio of the ocean may alter and diagenesis of the sedimentary coccoliths becomes an important consideration. *Ziveri et al.* [2000] have made a significant advance in developing a method for separating single species from a bulk sediment sample, but the challenge of translating these results from culture into application to the species- and time-integrated ocean sediments is still outstanding.

5. Implications for Biomineralization

[20] Previous studies suggest that calcification in *E. huxleyi* is related to photosynthetic carbon fixation [*Simkiss and Wilbur*, 1989; *Sikes and Fabry*, 1994; *Brownlee et al.*, 1995], although identifying the exact interactions by which the two processes are linked is still contentious [e.g., *McConnaughey*, 1993; *Brownlee et al.*, 1995]. How photosynthetic carbon fixation and the incorporation of Sr into the calcite coccolith are connected is an even more challenging question. By exploring the mechanism of biomineralization of coccolithophorids, it may be possible to answer this question and gain insight into why calcifying algae developed in the first place.

[21] A physical mechanism for the assembly and extrusion of a coccolith has been proposed by *Westbroek et al.* [1984] and detailed by *Simkiss and Wilbur* [1989] and *Lowenstam and Weiner* [1989]. The coccolith forms in a vesicle closely associated with the nucleus in *E. huxleyi*. Attached to this coccolith vesicle is a reticular body, which is thought to pass matrix material and calcium, supplied by Golgi vesicles, to the forming coccolith. Assembly of the *E. huxleyi* coccolith, which consists of a cycle of radially and vertically oriented calcite crystals, involves a folded acidic polysaccharide matrix [*Young et al.*, 1992]. This polysaccharide matrix is thought to promote and mould calcification by providing uranic acid groups as nucleation sites for Ca^{2+} but also inhibits crystal growth by adhering to the surface of the coccolith when construction is complete [*Westbroek et al.*, 1984]. Once the coccolith is formed, the reticular body detaches and degenerates while the coccolith vesicle moves to the surface of the cell and exocytoses the mineralized body onto the surface to form an interlocking sphere of coccoliths.

[22] As calcification occurs in an intracellular vesicle, it is likely that the biological control on the relative rates of supply of Ca^{2+} and Sr^{2+} ions to the vesicle outweigh any inorganic kinetic controls [*Lorens*, 1981] on the composition of the precipitating calcite. This is exemplified by the greater sensitivity of the cultured coccolith Sr/Ca to changes in growth rate compared to inorganic experiments, although absolute comparison of values is difficult because

of the inorganic data depending on the mass of seed added. In inorganic systems a 100-fold increase in precipitation rate is associated with a 3.5-fold increase in D_{Sr}, whereas in culture an increase in precipitation rate by only 1 order of magnitude results in a sixfold increase in D_{Sr}. All of the values of D_{Sr} published for coccolithophorids are higher than those quoted for inorganic systems, which implies that biology must be controlling the chemistry of the coccolith.

[23] In order to understand how Sr may be affected by the biomineralization process, it is necessary to review intracellular Ca and Sr transport. Calcium plays a dual role in *E. huxleyi* as both a substrate for calcification and an intracellular regulator, and the cytosol concentration of free calcium is rigorously controlled and maintained at a very low level. By contrast, Sr has no specific biological role in a coccolithophore. Sr is more chemically similar to Ca than any other trace element, and so it is possible that it substitutes for Ca and is transported via the same mechanism but at a different rate as Ca across each membrane. The Ca^{2+} ions necessary for the formation of coccoliths diffuse from seawater through Ca^{2+}-selective channels into the cytosol of the coccolithophore driven by a potential difference and by a very low calcium ion activity in the cytosol (0.1 μM). This low cytosolic concentration of Ca means that Ca must be pumped against a concentration gradient at some stage during its transport to the coccolith vesicle in order to attain saturation within the coccolith vesicle sufficient for the precipitation of calcite. The rate-dependent discrimination between the biological transport of Sr^{2+} and Ca^{2+} suggested by our data could be associated with passive transport through ion channels or active pumping via carrier proteins. It should be noted that in a biologically more complex hermatypic coral, the precipitation of Sr and Ca may involve different biochemical mechanisms [*Ip and Krishnaveni*, 1991].

[24] Carrier proteins, which transport Ca against a concentration gradient, act in a similar way to an enzyme-substrate reaction and have a binding site specific to the transported ion. One possibility is that the rate-dependent discrimination between Sr and Ca is controlled by the pumping of these specific carrier proteins. The carrier proteins could bind Ca^{2+} ions noncovalently more strongly than the Sr^{2+} ions because of the marginally higher charge density, as has been suggested by *Stephan and Hasselbach* [1991]. This means that the maximal rate of transport by the carrier protein V_{max} would be attained at a lower concentration of Ca than Sr (Figure 4). As the rate of transport increases, the concentration of transported Sr increases proportionally more than the Ca. Therefore the Sr/Ca transported to the vesicle and available for precipitation will increase with increased rates of pumping. This pumping of Ca against a concentration gradient requires an input of energy, which is most probably derived from photosynthetic products. In addition, a supplementary source of carbon dioxide for photosynthesis may be derived from the calcification reactions occurring within the coccolith vesicle such that higher ion pumping rates and calcification lead to higher rates of photosynthetic carbon fixation [*Sikes et al.*, 1980; *Sikes and Wheeler*, 1982; *Brownlee et al.*, 1995; *McConnaughey*, 1993]. These scenarios provide a plausible explanation for the relationship between μ_{PIC} and D_{Sr}.

[25] Alternatively, the rate-dependent discrimination between the transport of Sr and Ca may occur during diffusion through the Ca^{2+} channels. There are indications that cytosolic Ca^{2+} controls this diffusion and also regulates directly the uptake of the bicarbonate ion (HCO_3^-) [*Brownlee et al.*, 1995]. HCO_3^- can be considered to move passively into the cell, and at a pH of 7.0 a proportion will be protonated to form CO_2 for photosynthesis, and an equivalent amount is transported into the coccolith vesicle for calcification reactions [*Anning et al.*, 1996]. Therefore, at high rates of photosynthetic carbon fixation and calcification, more HCO_3^- is required by the coccolithophore. Assuming that cytosolic Ca^{2+} is regulating

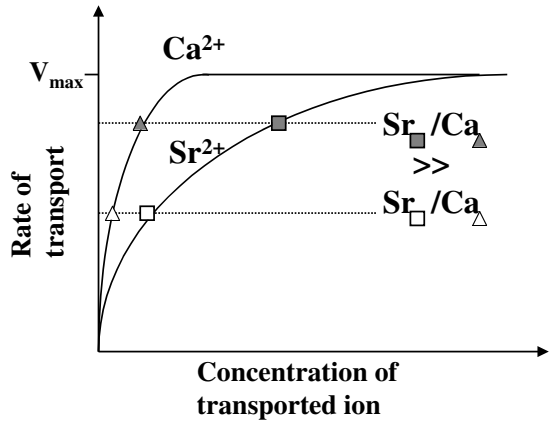

Figure 4. A hypothetical plot of rate of transport of an ion by a carrier protein versus the concentration of the transported ion for Ca^{2+} (triangles) and Sr^{2+} (squares). The slightly higher charge density of the calcium ion relative to the strontium ion leads to stronger bonding of the calcium ion by the protein and more efficient transport at lower concentrations. As the rate of transport of ions increases from the open symbols to the shaded symbols, the Sr/Ca of the transported ions will also increase as marked by the dotted lines.

HCO_3^- transport, then increased HCO_3^- could be achieved by an increased rate of supply of Ca through the ion pumps. If the Ca^{2+}-channels transport ions more quickly, the selectivity against Sr may become less efficient such that the Sr/Ca of the cytosol and, ultimately, the coccolith could increase accordingly.

[26] Each of the above mechanisms can be considered as a biological analogue of the inorganic model proposed by *Lorens* [1981]. In his model he proposes a rate-dependent discrimination by the crystal against the larger ions. We propose that strontium incorporation into coccoliths is controlled by a rate-dependent discrimination by the cell against the biological pumping of the larger ions.

6. Conclusions

[27] Data from controlled batch culture experiments confirm the initial proposal of *Stoll and Schrag* [2000] that the Sr/Ca of coccoliths is related to growth rate and calcification rate. There exists a striking correlation between μ_{PIC}, the Sr/Ca of coccoliths, and the rate of carbon fixation. We suggest that the rate control on the Sr/Ca of the coccoliths cannot be accounted for by predictions from inorganic experiments and that the biology of coccolithophores must dominate the composition of the ion supply to the intracellular coccolith-producing vesicle and ultimately the composition of the coccoliths. Two biological mechanisms are proposed to account for the intricate relationship between carbon fixation, calcification, and the incorporation of Sr^{2+} into coccoliths. Further experimentation on other trace elements is necessary to try and elucidate the selectivity of ion pumping during calcification. Finally, our empirically derived relationship between D_{Sr} and carbon-specific growth rate shows potential for a combined geochemical proxy approach, using Sr/Ca and ε_p, for reconstructing paleo-pCO_2.

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References

Anning, T., N. Nimer, M. J. Merrett, and C. Brownlee, Costs and benefits of calcification in coccolithophorids, *J. Mar. Syst.*, *9*, 45–46, 1996.

Balch, W. M., and K. Kilpatrick, Calcification rates in the equatorial Pacific along 140W, *Deep Sea Res., Part II*, *43*, 971–1003, 1996.

Bidigare, R. R., et al., Consistent fractionation of C-13 in nature and in the laboratory: Growth-rate effects in some haptophyte algae, *Global Biogeochem. Cycles*, *11*, 279–292, 1997.

Bidigare, R. R., et al., Iron-stimulated changes in ^{13}C fractionation and export by equatorial Pacific phytoplankton: Toward a paleogrowth rate proxy, *Paleoceanography*, *14*, 589–595, 1999.

Bradshaw, A. L., P. G. Brewer, D. K. Shafer, and R. T. Williams, Measurements of total carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program, *Earth Planet. Sci. Lett.*, *55*, 99–115, 1981.

Brewer, P. G., A. L. Bradshaw, and R. T. Williams, Measurements of total carbon dioxide and alkalinity in the North Atlantic Ocean in 1981, *The Changing Carbon Cycle: A Global Analysis*, edited by J. R. Trabalka and D. E. Reichle, pp. 592–695, Springer-Verlag, New York, 1986.

Broerse, A. T. C., Coccolithophore export production in selected ocean environments: Seasonality, biogeography and carbonate production, Ph.D. thesis, Vrije Univ., Amsterdam, 2000.

Brownlee, C., M. Davies, N. Nimer, L. F. Dong, and M. J. Merrett, Calcification, photosynthesis, and intracellular regulation in *Emiliania huxleyi*, *Bull. Inst. Oceanogr.*, *14*(2), Spec., 19–35, 1995.

Burkhardt, S., U. Riebesell, and I. Zondervan, Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton, *Geochim. Cosmochim. Acta*, *63*, 3729–3741, 1999.

De Villieurs, S., Seawater strontium and Sr/Ca variability in the Atlantic and Pacific Oceans, *Earth Planet. Sci. Lett.*, *171*, 623–634, 1999.

Dudley, W. C., and C. S. Nelson, The influence of nonequilibrium isotope fractionation on the Quaternary calcareous nannofossil stable-isotope signal in the southwest Pacific Ocean, DSDP Site 594, *Mar. Micropaleontol.*, *24*, 3–27, 1994.

Farquhar, G. D., M. H. O'Leary, and J. A. Berry, On the relationship between carbon isotope discrimination and the intracellular carbon dioxide concentration in leaves, *Aust. J. Plant Physiol.*, *9*, 121–137, 1982.

Francois, R. F., M. A. Altabet, R. Goericke, D. C. McCorkle, C. Brunet, and A. Poisson, Changes in the $\delta^{13}C$ of surface water particulate organic matter across the subtropical convergence in the SW Indian Ocean, *Global Biogeochem. Cycles*, *7*, 627–644, 1993.

Freeman, K. H., and J. M. Hayes, Fractionation of isotopes by phytoplankton and estimates of ancient CO_2 levels, *Global Biogeochem. Cycles*, *6*, 185–198, 1992.

Goericke, R., J. P. Montoya, and B. Fry, Physiology of isotopic fractionation in algae and cyanobacteria, in *Stable Isotopes in Ecology and Environmental Sciences,* edited by K. Lajtha and R. H. Michener, pp. 187–221, Blackwell Sci., Malden, Mass., 1994.

Goyet, C., and A. Poisson, New determination of carbonic-acid dissociation-constants in seawater as a function of temperature and salinity, *Deep Sea Res.*, *36*, 1635–1654, 1989.

Gran, G., Determination of the equivalence point in potentiometric titration, part II, *Analyst*, *77*, 661–671, 1952.

Guillard, R. R. L., and J. H. Ryther, Studies of marine plankton diatoms, in *Cyclotella nana*. Hustedt and *Detonula confervacea* (Cleve) Gran, *Can. J. Microbiol.*, *8*, 229–239, 1962.

Ip, Y. K., and P. Krishnaveni, Incorporation of strontium ($^{90}Sr^{2+}$) into the skeleton of the hermatypic coral *Galaxea fascicularis*, *J. Exp. Zool.*, *258*, 273–276, 1991.

Jasper, J. P., and J. M. Hayes, A carbon isotope record of CO_2 levels during the late Quaternary, *Nature*, *347*, 462–464, 1990.

Jasper, J. P., J. M. Hayes, A. C. Mix, and F. G. Prahl, Photosynthetic fractionation of ^{13}C and concentrations of dissolved CO_2 in the central equatorial Pacific during the last 255,000 years, *Paleoceanography*, *9*, 781–798, 1994.

Johnson, K. M., A. E. King, and J. M. Sieburth, Coulometric TCO_2 analyses for marine studies: An introduction, *Mar. Chem.*, *16*, 61–82, 1985.

Laws, E. A., and T. T. Bannister, Nutrient-limited and light-limited growth of *Thalassiosira-Fluviatis* in continuous culture, with implications for phytoplankton growth in the ocean, *Limnol. Oceanogr.*, *25*, 457–473, 1980.

Laws, E. A., B. N. Popp, R. B. Bidigare, M. C. Kennicutt, and S. A. Macko, Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental: Results, *Geochim. Cosmochim. Acta*, *59*, 1131–1138, 1995.

Laws, E. A., B. N. Popp, R. R. Bidigare, U. Riebesell, S. Burkhardt, and S. G. Wakeham, Controls on the molecular distribution and carbon isotopic composition of alkenones in certain haptophyte algae, *Geochem. Geophys. Geosyst.*, vol. 2, Paper number 2000GC000057 [15,290 words, 7 figures, 3 tables]. Published January 25, 2001.

Lorens, R. B., Sr, Cd, Mn and Co distribution coefficients in calcite as a function of calcite precipitation rate, *Geochim. Cosmochim. Acta*, *45*, 553–561, 1981.

Lowenstam, H. A., and S. Weiner, *On Biomineralization*, Oxford Univ. Press, New York, 1989.

McConnaughey, T., ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates, 1, Patterns, *Geochim. Cosmochim. Acta*, *53*, 151–162, 1989.

McConnaughey, T., Ion transport and the generation of biomineral supersaturation, *Bull. Inst. Oceanogr.*, *14*(2), Spec., 1–18, 1993.

Mook, W. G., J. C. Bommerson, and W. H. Staverman, Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide, *Earth Planet. Sci. Lett.*, *22*, 169–176, 1974.

Morse, J. W., and M. L. Bender, Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems, *Chem. Geol.*, *82*, 265–277, 1990.

Paasche, E., and S. Brubak, Enhanced calcification of the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorous limitation, *Phycologia*, *33*, 324–330, 1994.

Pagani, M., K. H. Freeman, and M. A. Arthur, Late Miocene atmospheric CO_2 concentrations and the expansion of C_4 grasses, *Science*, *285*, 876–879, 1999.

Petit, J. R., et al., Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica, *Nature*, *399*, 429–436, 1999.

Popp, B. N., E. A. Laws, R. R. Bidigare, J. E. Dore, K. L. Hanson, and S. G. Wakeham, Effect of phytoplankton cell geometry on carbon isotopic fractionation, *Geochim. Cosmochim. Acta*, *62*, 69–77, 1998.

Popp, B. N., K. L. Hanson, J. E. Dore, R. R. Bidigare, E. A. Laws, and S. G. Wakeham, Controls on the carbon isotopic composition of phytoplankton: Paleoceanographic perspectives, in *Reconstructing Ocean History: A Window Into the Future*, edited by A. Abrantes and A. Mix, pp. 381–398, Kluwer Acad., Norwell, Mass., 1999.

Rau, G. H., U. Riebesell, and D. Wolf-Gladrow, A model of photosynthetic ^{13}C fractionation by marine phytoplankton based on diffusive molecular CO_2 uptake, *Mar. Ecol. Prog. Ser.*, *133*, 275–285, 1996.

Rickaby, R. E. M., and H. Elderfield, Planktonic foraminiferal Cd/Ca: Paleonutrients or paleotemperature?, *Paleoceanography*, *14*, 293–303, 1999.

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, and F. M. M. Morel, Reduced calcification of marine plankton in response to increased atmospheric CO_2, *Nature*, *407*, 364–367, 2000a.

Riebesell, U., S. Burkhardt, A. Dauelsberg, and B. Kroon, Carbon isotope fractionation by a marine diatom: dependence on the growth-limiting resource, *Mar. Ecol. Prog. Ser.*, *193*, 295–303, 2000b.

Rosenthal, Y., M. Dahan, and A. Shemesh, Southern Ocean contributions to glacial-interglacial changes of atmospheric pCO_2: An assessment of carbon isotope records in diatoms, *Paleoceanography*, *15*, 65–76, 2000.

Schrag, D. P., Rapid analysis of high-precision Sr/Ca ratios in corals and other marine carbonates, *Paleoceanography*, *14*, 97–102, 1999.

Sikes, C. S., and V. J. Fabry, Photosynthesis, CaCO3 deposition, coccolithophorids and the global carbon cycle, *Photosynthetic Carbon Metabolism and Regulation of Atmospheric CO_2 and O_2*, edited by N. E. Tolbert and J. Priess, pp. 217–233, Oxford Univ. Press, New York, 1994.

Sikes, C. S., and A. P. Wheeler, Carbonic anhydrase and carbon fixation in coccolithophorids, *J. Phycol.*, *18*, 123–126, 1982.

Sikes, C. S., R. D. Roer, and K. M. Wilbur, Photosynthesis and coccolith formation: Inorganic carbon sources and net inorganic reaction of deposition, *Limnol. Oceanogr.*, *25*, 248–261, 1980.

Simkiss, K., and K. M. Wilbur, *Biomineralization: Cell Biology and Mineral Deposition*, Academic, San Diego, Calif., 1989.

Stephan, S., and W. Hasselbach, Activation and binding volumes of the sarcoplasmic reticulum transport enzyme activated by calcium or strontium, *Eur. J. Biochem.*, *196*, 231–237, 1991.

Stoll, H. M., and D. P. Schrag, Effects of Quaternary Sea level changes on strontium in seawater, *Geochim.. Cosmochim. Acta*, *62*, 1107–1118, 1998.

Stoll, H. M., and D. P. Schrag, Coccolith Sr/Ca as a new indicator of coccolithophorid calcification and growth rate, *Geochem. Geophys. Geosyst.*, vol. 1, Paper number 1999GC000015 [12,215 words, 12 figures, 2 tables]. May 30, 2000.

Stoll, H. M., C. M. Klaas, and I. Probert, Calcification and temperature effects on Sr partitioning in coccoliths of multiple species of coccolithophorids in culture, *Global Planet. Change*, in press, 2001.

Tortell, P. D., J. R. Reinfelder, and F. M. M. Morel, Active uptake of bicarbonate by diatoms, *Nature*, *390*, 243–244, 1997.

Tortell, P. D., G. H. Rau, and F. M. M. Morel, Inorganic carbon acquisition in coastal Pacific phytoplankton communities, *Limnol. Oceanogr.*, *45*, 1485–1500, 2000.

Tesoriero, A. J., and J. F. Pankow, solid solution partitioning of Sr^{2+}, Ba^{2+}, and Cd^{2+} to calcite, *Geochim. Cosmochim. Acta*, *60*, 1053–1063, 1996.

Van Bleijswijk, J. D. L., R. S. Kempers, M. J. Veldhuis, and P. Westbroek, Cell and growth characteristics of type A and type B of *Emiliania huxleyi* (Prymnesiophyceae) as determined by flow cytometry and chemical analyses, *J. Phycol.*, *30*, 230–241, 1994.

Watson, E. B., Surface enrichment and trace-element uptake during crystal growth, *Geochim. Cosmochim. Acta*, *60*, 5013–5020, 1996.

Westbroek, P., et al., Mechanism of calcification in the marine alga *Emiliania huxleyi*, *Philos. Trans. R. Soc. London, Ser. B*, *304*, 435–444, 1984.

Young, J. R., J. M. Didymus, P. R. Bown, B. Prins, and S. Mann, Crystal assembly and phylogenetic evolution in heterococcoliths, *Nature*, *356*, 516–518, 1992.

Ziveri, P., I. Probert, C. Klaas, H. M. Stoll, G. M. Ganssen, and J. R. Young, Species-specific stable isotope composition of coccolith calcite as a paleoceanographic proxy, *Eos Trans. AGU*, *81*(48), Fall Meet. Suppl., 2000.

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