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Potential and limitations of Sr/Ca ratios in coccolith carbonate: new perspectives from cultures and monospecific samples from sediments

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The Sr/Ca ratio of coccoliths was recently proposed as a potential indicator of past growth rates of coccolithophorids, marine algae, which play key roles in both the global carbonate and carbon cycles. We synthesize calibrations of this proxy through laboratory culture studies and analysis of monospecific coccolith assemblages from surface sediments. Cultures of coccolithophorids *Helicosphaera carteri*, *Syracosphaera pulchra* and *Algirospira robusta* confirm a 1–2% increase in Sr/Ca per °C previously identified in *Emiliania huxleyi* and *Gephyrocapsa oceanica*. This effect is not due merely to increases in growth rate with temperature and must be considered in palaeoceanographic studies. In light-limited cultures of *E. huxleyi*, *Calcidiscus leptoporus* and *G. oceanica* at constant temperature, coccolith Sr/Ca ratios vary by 10% across the range of possible growth and calcification rates for a given species. Among different species under similar culture conditions, Sr/Ca ratios vary by 30%. Although the highest ratios are in the cells with highest calcification and organic carbon fixation rates, at lower rates there is much scatter, indicating that different mechanisms control interspecific and intraspecific coccolith Sr/Ca variations. In field studies in the Equatorial Pacific and Somalia coastal region, coccolith Sr/Ca correlates with upwelling intensity and productivity. A more dynamic response is observed in larger coccoliths like *C. leptoporus* (23–55% variation in Sr/Ca) than in smaller coccoliths of *G. oceanica* or *Florisphaera profunda* (6–15% variation in Sr/Ca). This response suggests that, despite temperature effects, coccolith Sr/Ca has potential as an indicator of coccolithophorid productivity. If the variable Sr/Ca response of different species accurately reflects their variable productivity response to upwelling (and not different slopes of Sr/Ca with productivity), coccolith Sr/Ca could provide useful data on past changes in coccolith ecology. The mechanism of coccolith Sr/Ca variations remains poorly understood but is probably more closely

tied to biochemical cycles during carbon acquisition than to chemical kinetic effects on Sr incorporation in the calcite coccolith crystals.

Keywords: coccoliths; coccolithophorids; trace element; strontium; Sr/Ca ratio; palaeoproductivity

1. Introduction

Characterizing relationships between the carbon cycle and climate change, especially understanding how changes in the marine productivity may drive short-term changes in atmospheric CO_2 (Archer *et al*. 2000), is of increasing importance in palaeoclimate studies. In particular, there is rising interest in understanding the past responses of different ecological groups (carbonate versus silica producers) to climate change (Archer *et al*. 2000). However, to date there are a limited number of proxies that reveal past changes in the carbon cycle, particularly at the level of ecological groups.

The geochemistry of coccolithophorids may offer one such palaeoproxy. These marine algae are key players in the global carbonate cycle, and are probably the most important carbonate producers in open ocean settings (Takahashi 1994; Westbroek *et al*. 1994; Archer *et al*. 2000). Coccolithophorids occur throughout the ocean, from subpolar to tropical regions, and the calcite plates or coccoliths they produce to cover the cell exterior are well preserved in the marine sediment record. Blooms of some species, especially *Emiliania huxleyi*, occur annually in high latitudes, but in most areas of the ocean coccolithophorid productivity is from relatively modest standing stocks of diverse larger species. In fact, while *E. huxleyi* may be the most numerous species in many photic-zone regions, it is rarely the dominant contributor to coccolith carbonate in sediments. Significant contributors to coccolith carbonate in sediments are *Coccolithus pelagicus* at high latitudes, and *Gephyrocapsa oceanica*, *Calcidiscus leptoporus* and *Helicosphaera carterae* at low latitudes (see figure 1; Young & Ziveri 2000).

The Sr/Ca ratio of coccoliths was recently proposed as a potential indicator of past growth rates of coccolithophorids on the basis of correlations of Sr/Ca ratios in polyspecific coccolith samples with, variously, primary productivity, alkenone-estimated growth rates and $CaCO_3$ rain rates in deep sediment traps (see, for example, Stoll & Schrag 2000) across the Equatorial Pacific upwelling region. Subsequently, a number of culture studies have investigated controls on Sr/Ca ratios in coccoliths of a number of species. If coccolith Sr/Ca is a reliable indicator of coccolithophorid productivity, it would have the advantage of providing a record of past productivity variations directly from a primary producer. Furthermore, unlike many other mass-flux-derived productivity estimates, estimates from coccolith Sr/Ca would not rely on determination of sediment accumulation rates, which are frequently imprecise. Coccolith Sr/Ca ratios also appear to be relatively insensitive to partial dissolution (Stoll & Schrag 2000).

Here, we integrate new results from cultures and field studies with results from recent culture studies. Laboratory cultures represent a useful model system in which to explore controls over the geochemistry of coccoliths. The relative ease with which coccolith calcite may be produced in culture has led to much more rapid advances in understanding control over coccolith chemistry than in other calcifying organisms. To test whether growth rate controls coccolith Sr/Ca, culture experiments can

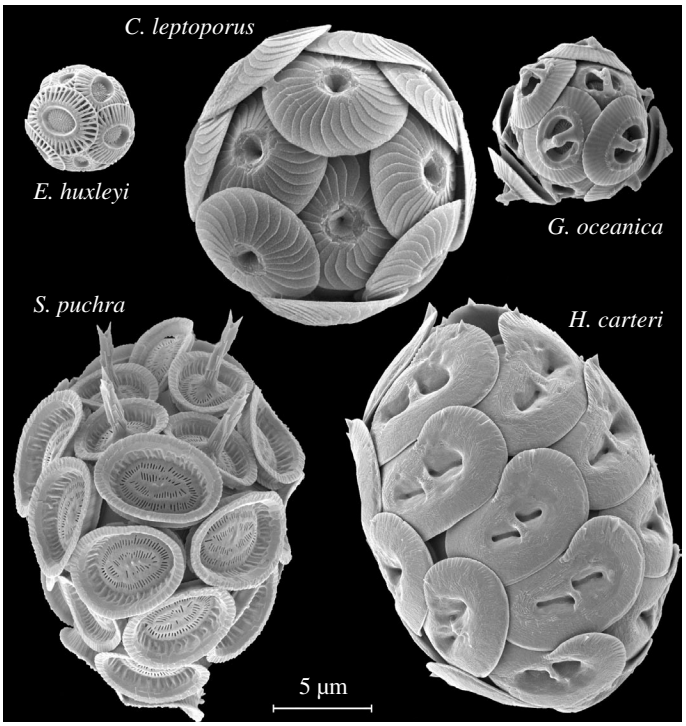

Figure 1. Scanning electron micrographs of coccospheres of the principal species discussed here: *Emiliania huxleyi*, *Calcidiscus leptoporus*, *Gephyrocapsa oceanica*, *Syracosphaera pulchra* and *Helicosphaera carteri*. All species are from plankton samples and illustrated at the same scale. Note the size range of the species; the larger species are numerically less abundant but have much higher preservation potential and frequently contribute the bulk of carbonate. Note also the morphological diversity of the species; taken together, these species form a broad sampling of the phylogenetic and ecological diversity of the coccolithophores.

most readily regulate growth rate via changes in culture temperature or light levels. These factors may not necessarily be those that most strongly limit coccolithophorid growth rates in most natural oceanic settings. In the ocean, coccolithophores are not likely to be light limited, except in highly productive settings where turbidity reduces light penetration. Regulating growth rate by factors that likely limit coccolithophorid growth in the ocean (nutrient (and micronutrient) concentrations, and possibly carbon availability) is more difficult in cultures. However, if growth and/or calcification rate is the ultimate control over coccolith Sr/Ca, we should see a uniform response regardless of what factor controls growth rate.

Calibrations of proxies in natural or field settings may be the most reliable method, since the environment is closest to that in which we wish to apply the palaeoproxy. While this approach has proved valuable for a number of proxies, especially those reconstructing sea surface temperature (like U^k_{37}), there are additional challenges in trying to understand relationships about coccolith Sr/Ca. Measurements of primary productivity, as opposed to standing stocks, in the oceans are few, and measurements of primary productivity of specific groups of primary producers (like haptophytes or coccolithophores) are even more scarce. To date, new techniques to measure productivity of individual groups, based on diel changes in DNA content (van Bleijwsijk

Table 1. *Recent culture experiments for chemistry of coccoliths*

culture lab experiment	species (no. strains)	type	media and nutrients (μM NO_3^-)	light (μE m^{-2} s^{-1})	light: dark (h)	temp. (°C)	CO_2 in culture atm.	harvest method
ETH	*G. oceanica* (6) *C. leptoporus* (1)	batch	f/2 (884)	saturated (175 ± 25)	14:10	17–30	ambient	centrifuge
Caen (I)	*G. oceanica* (1) *C. leptoporus* (1) *E. huxleyi* (2) *C. pelagicus pel.* (1) *U. sibogae* (2)	batch	k/2 (884)	saturated	14:10	17	ambient	centrifuge
Rutgers	*E. huxleyi* (1)	continuous	f/2 (884), f/50 (35)	variable (9–146)	24	7–25	ambient	centrifuge
AWI	*E. huxleyi* (1)	batch (mesocosm)	f/2 for phosphate, vitamins and metals, NO_3^- 0.3–1.9 μM	gradient (65–125)	16:08	14.6 (±0.5)	500–700 ppmv	glass fibre filter
Caen (II)	*C. pelagicus hyal.* (1) *A. robusta* (1) *O. fragilis* (1) *S. pulchra* (1) *U. hurlburtiana* (1)	batch	k/2 (884)	saturated	14:10	17	ambient	centrifuge
Caen (III)	*G. oceanica* (1) *C. leptoporus* (1)	batch	k/2 (884)	variable (15, 60, 137)	14:10	17	ambient	centrifuge
Caen (IV)	*E. huxleyi* (1) *G. oceanica* (1) *A. robusta* (1)	batch	k/2 (884)	saturated	14:10	26.5	ambient	centrifuge
NHM	*S. pulchra* (2) *H. carterae* (2)	batch	k/2 (884)	saturated	14:10	13.8–23.8	ambient	centrifuge

Table 1. *Cont.*

culture lab experiment	cleaning for Sr/Ca analysis	reference	major result or test on Sr/Ca (% variation in Sr/Ca ratio)
ETH	rinses in distilled water and methanol	Stoll *et al.* (2001, 2002*a*)	Sr/Ca increases (30%) with growth rate and/or temperature (30%)
Caen (I)	rinses in distilled water and ethanol	Stoll *et al.* (2001, 2002*a*)	interspecific Sr/Ca differences (30%) correlate with calcification rate and organic C fixation rate
Rutgers	rinses in distilled water and ethanol, oxidative cleaning on splits	Stoll *et al.* (2002*a*)	larger Sr/Ca variations with temperature (25%) than over comparable range in growth rate at constant temperature (10%); smaller Sr/Ca variations with growth rate than in Equatorial Pacific field study
AWI	oxidation in NaOCl, rinse in ethanol	Rickaby *et al.* (2002)	
Caen (II)	rinses in distilled water and ethanol	new data	test further range of interspecific effects in Sr/Ca
Caen (III)	rinses in distilled water and ethanol	new data	test growth rate effects at constant temperature in other species
Caen (IV)	rinses in distilled water and ethanol	new data	test temperature effects in other species
NHM	rinses in distilled water and ethanol, some samples only ethanol	new data	test temperature effects in other species

1996), ^{14}C labelling of specific cartenoid pigments (Gieskes & Kraay 1989), or C-isotopic fractionation in alkenones (Bidigare *et al*. 1997), have been applied only in a few test regions. Consequently, while it is relatively easy to measure Sr/Ca in coccoliths from sediment core tops, it is harder to find data on surface conditions in which they likely grew. The most informative field studies have focused on transects across upwelling regions, where there are strong and easily identifiable gradients in primary productivity. These smaller scale transects also offer the advantage of a relatively constant assemblage of coccolith species.

New experiments presented here were designed to investigate several key questions raised by earlier experiments, especially the relative role of growth rate and temperature in controlling coccolith Sr/Ca and how these relative contributions may vary among different species. The new field data provide the first look at coccolith Sr/Ca in monospecific samples from surface sediments, comparing responses in different species, applying a new method for separation of near-monospecific coccolith samples from sediments (Stoll & Ziveri 2002). These data allow us to evaluate several questions key to successful palaeoceanographic applications of coccolith Sr/Ca.

(i) Do cultures give self-consistent results?

(ii) Are there species-specific responses of coccolith Sr/Ca to growth rate variations in cultures or in the same sediments?

(iii) Are culture results consistent with field results?

(iv) How well do we understand the mechanism of coccolith Sr/Ca dependence on growth or calcification rate?

We also briefly review recent advances in coccolith proxies from Mg/Ca and stable isotope ratios.

2. Methods

(*a*) *Culture data*

Recent culture experiments used to study coccolith chemistry are summarized in table 1. Here we present new results from cultures of *G. oceanica* and *C. leptoporus*, where growth rate is limited by irradiance. We also present the first (to our knowledge) Sr/Ca culture data from the species *Helicosphaera carteri* and *Syracosphaera pulchra*. These four species are representatives of the four major clades present in the modern oceanic coccolithophorid community and are believed to have diverged in the Mesozoic (Young *et al*. 1999; A. Saez, unpublished molecular genetic data). Hence they span the phylogenetic diversity of modern cocolithophorids and it is reasonable to hypothesize that common patterns shown by them will hold for occolthophorids in general. In addition, *H. carteri* can be a major contributor of coccolith carbonate in oligotrophic areas (Stoll & Ziveri 2002). These cultures, together with new cultures of *Algirosphaera robusta* and *G. oceanica*, provide information on the influence of temperature in key species.

(i) *Culture set-up*

For the new batch of culture experiments described here, samples were taken during a single growth cycle, during which cell densities progressively increased, resulting

in changes in media pH, dissolved inorganic carbon and nutrient concentrations. Cultures were grown in 300 ml batch cultures in filter-sterilized coastal sea water (Caen cultures of table 1) or autoclaved coastal sea water (NHM cultures of table 1) enriched in nutrients, vitamins and trace metals to standards for K/2 medium (Keller *et al.* 1987) in light-saturating conditions on a 14:10 LD cycle. In all experiments, cultures were acclimatized to the experimental conditions (growth medium, temperature and light) for at least two weeks (greater than 10 cell divisions) before inoculation. Cultures were sampled in the log phase except where noted. Log-phase growth rates for each culture experiment are calculated from frequent cell-density measurements with a haemocytometer. Where cells were harvested in the early stationary phase, we report growth rates based on cell counts during the exponential phase only. We estimate the rate of organic C uptake by the cells from calculating organic C quotas of the cells times the cell division rate. We assume that the organic C quota is proportional to biovolume and use relationships from experiments with *E. huxleyi* indicating typical cell quotas of 20 pg organic C/cell with cell diameters of *ca.* 4μm (Fernandez *et al.* 1997). Cell diameters were measured with an eyepiece graticule during the culture experiments.

We focus here on two series of experiments. In one, *S. pulchra* and *H. carteri* were grown at constant saturated irradiance and temperatures ranging from 13.8 to 23.8 °C. For the temperature experiment with *S. pulchra* and *H. carteri*, two strains were cultured but samples were taken from each culture at only one point in their growth curve. While the batch cultures of most species were all harvested in the exponential phase for *H. carteri*, all but the coolest two temperatures were collected in early stationary phase. For both *H. carteri* and *S. pulchra*, growth rates were generally higher at higher temperatures. Since cultures were all harvested on the same day, higher-temperature cultures were sampled at higher cell densities.

In another experiment, cultures of *C. leptoporus* and *G. oceanica* were grown at constant temperature but three irradiance levels:

$$15\ (\pm 5), \quad 60\ (\pm 10) \quad \text{and} \quad 137\ (\pm 13)\ \mu\text{E}\ \text{m}^{-2}\ \text{s}^{-1}.$$

For these experiments, two replicates of a single strain were cultured. From each culture, samples were harvested at low cell densities and high cell densities, both in logarithmic growth phase. In addition to these light and variable temperature experiments, a number of additional species were cultured at 17 °C and light-saturating conditions to further investigate interspecific differences in coccolith Sr/Ca ratios. Ten to thirty millilitres of culture was centrifuged down to concentrate cells. Calcite production of the cells in culture was estimated by dissolving the harvested coccolith pellet and measuring the Ca concentration via flame atomic absorption spectroscopy. For Sr/Ca analysis of coccoliths, the importance of different cleaning approaches was evaluated by comparing Sr/Ca ratios of distilled water- and ethanol-rinsed samples. Sr/Ca ratios were measured via inductively coupled argon plasma spectrometry in axial mode (thermo elemental model IRIS 1000 DUO at Middlebury College, Vermont, USA). Samples were analysed at concentrations ranging from 10 to 30 ppm Ca. Measured ratios on standards were independent of concentration or strength of acid matrix (0.2–2% HNO_3). Precision, based on r.s.d. of replicate samples throughout the run, averages better than 0.5%.

(ii) *Cleaning culture samples*

Typically, culture samples are rinsed with distilled water by the culture laboratory immediately after harvest, and then cleaned in successive rinses of ethanol and distilled water in geochemical laboratories. For example, for the *C. leptoporus* and *G. oceanica* cultures, the pellet was rinsed twice in distilled water (with pH adjusted to 8.5 with NH_4OH), twice in ethanol, and twice with distilled water before dissolution in 50 µl 2% HNO_3 for analysis. The *S. pulchra* and *H. carteri* culture pellets were rinsed in ethanol, rather than water, immediately after harvest. In the geochemical laboratory they were rinsed again in ethanol as the first step in the series of rinse steps prior to dissolution. However, during the subsequent rinse of these samples in 1.5 ml distilled water (with pH adjusted to 8.5 with NH_4OH), significant material dissolved during the rinse step (as much as half of the sample). We measured the Sr/Ca ratios of rinse solutions, samples which had been rinsed in only ethanol, and samples rinsed in distilled water, to compare the effect of this different cleaning.

Sr/Ca ratios in most distilled-water rinses of *S. pulchra* and *H. carteri* ranged from 7.4 to 7.9 mmol mol^{-1}; two samples had lower ratios of 5.6 and 6.9 mmol mol^{-1}. Na/Ca ratios confirm that the high Sr/Ca ratios in the rinse solutions do not result from contamination with sea water (less than 8% of the Ca in the rinse solution could derive from sea salt, which would cause only a 0.1 mmol mol^{-1} elevation of the Sr/Ca ratios in the rinse). Rinsed *S. pulchra* or *H. carteri* samples had much lower Sr/Ca ratios (2.3–3.16 mmol mol^{-1}). Sr/Ca ratios from unrinsed samples had intermediate Sr/Ca ratios (3.5–5.2 mmol mol^{-1}). These Sr/Ca ratios imply a very large heterogeneity in compositions between fractions that are more soluble (released in distilled-water rinse) versus less soluble (surviving the distilled-water rinse) (figure 2*a*). Scanning electron microscope (SEM) images show the distilled-water-rinsed samples contain only coccoliths, whereas the ethanol-rinsed samples contain coccoliths along with many small unidentified needle-like particulates. Since the appearance of the coccoliths is identical in both samples, we infer that the small particulates are the highly soluble phase. The high amount of Ca released in the rinse solutions (equivalent to 4–40 µg Ca, representing as much as half the Ca present in each sample), coincident with the loss of white powder, suggests that this highly soluble phase is carbonate. The high Sr/Ca ratios are much more typical of aragonite than calcite.

The amount of Ca liberated in the rinse step varied among the samples, with the lowest amount dissolved in the samples with highest total Ca content (figure 2*b*). This suggests that partial dissolution in these samples is not a continuous process limited by saturation but a step-wise process limited by the kinetics of dissolution. There appears to be a finite amount of higher Sr/Ca, highly soluble phase present in each sample, all of which is dissolved in the rinse step (figure 2*c*). The similarity in Sr/Ca ratios of most of the rinses (despite variable amounts of carbonate dissolved in rinses) may indicate that little of the lower Sr/Ca, less soluble carbonate fraction, is dissolved in this first rinse step (or that the amount dissolved is relatively constant).

For *H. carteri*, the proportion of highly soluble Ca phase (dissolving in the rinse) was inversely proportional to the cell density at culture harvest. In samples collected at higher cell densities, a large fraction of this more soluble phase may have already dissolved in the culture, since dissolution of carbonate in the culture increases with cell density as media Ca concentrations and alkalinity decrease (figure 2*d*). Because of this decreased proportion of highly soluble phase with cell density, there is a strong

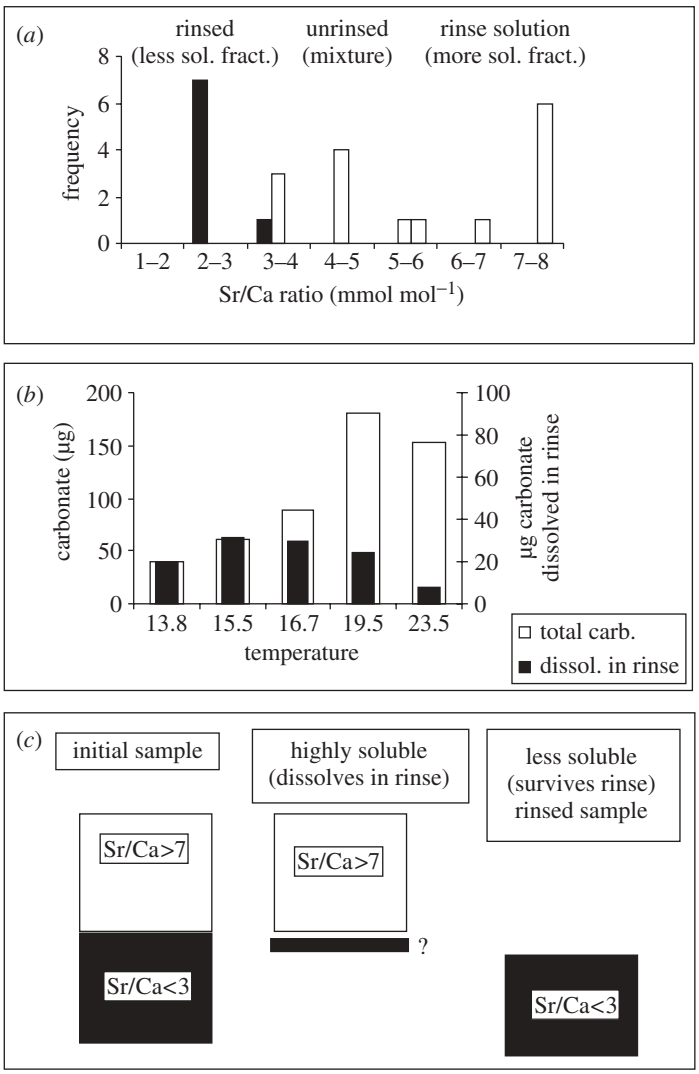

Figure 2. (*a*) Histogram of Sr/Ca ratios of different fractions of culture samples from *H. carteri* and *S. pulchra* released in distilled-water rinse solutions, in samples dissolved in HNO_3 after rinsing, and in samples dissolved directly with no rinsing. (*b*) Amount of Ca released in distilled water rinses of samples of *H. carteri* NS10-8, compared with total amount of Ca in the sample. Note that scales differ by a factor of two. Amounts represent Ca harvested from 10 ml samples of culture. (*c*) Interpretation of heterogeneous composition of culture sample and its dissolution during cleaning.

inverse relationship between cell density and the Sr/Ca of unrinsed samples, which represents a mixture of the two compositions (figure 2*e*).

Our previous culture and field studies have not revealed a comparable level of heterogeneity in culture samples, probably because the highly soluble phase, if present, was removed prior to analysis. Experiments with *E. huxleyi* and *C. leptoporus* showed that Sr/Ca ratios were similar for samples with minimal and more extensive rinsing/cleaning steps (Stoll *et al.* 2002*a*, *b*), but all had been rinsed at least once in

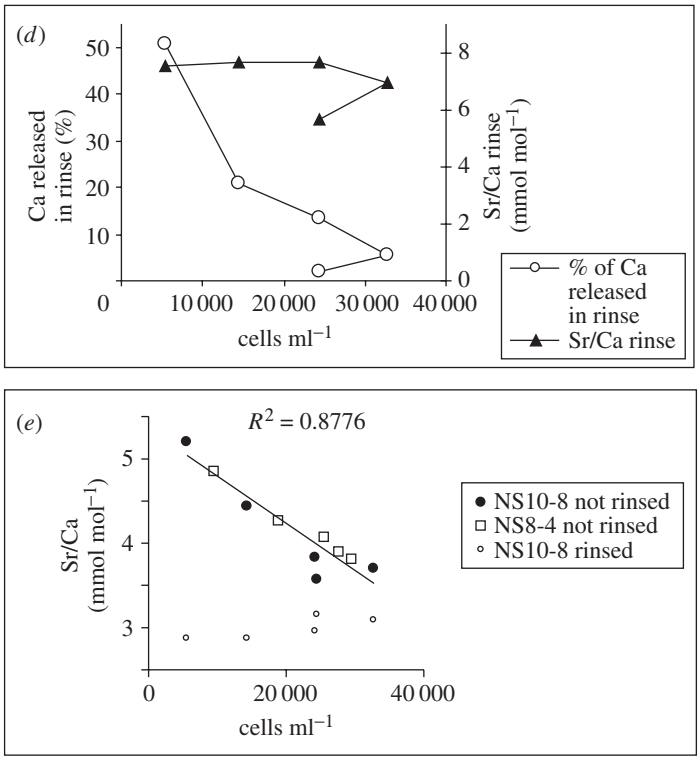

Figure 2. (*Cont.*) (*d*) Decrease in fraction of highly soluble phase (Ca released in rinse, triangles) vs Sr/Ca released in rinse (open circles). The sample with the lowest percentage of highly soluble phase is from the 23 °C experiment, which was harvested more than 10 days after reaching stationary phase (all others were harvested in exponential phase). (*e*) Sr/Ca ratio of solid phase in unrinsed (filled circle, open square) and rinsed (open circle) sample as a function of cell density.

distilled water. The *E. huxleyi* culture data of Rickaby *et al*. (2002) may provide the only other evidence of the highly soluble Sr phase. These samples were rinsed only in ethanol and bleach (whose high pH tends to promote overgrowths, rather than dissolution (Bairbakesh *et al*. 1999)). Sr/Ca ratios of *E. huxleyi* coccoliths decreased from nearly 6 to less than 2 mmol mol^{-1} through logarithmic growth phase as the cell density increased from 3000 to 45 000 cells ml^{-1}. As was the case for our unrinsed *H. carteri* samples, Sr/Ca of the *E. huxleyi* samples was highly inversely correlated with cell density. It is possible that the decrease in Sr/Ca ratio through time in these cultures was a consequence of progressive removal of a highly soluble Sr-rich phase by dissolution in the culture.

Since the highly soluble high-Sr/Ca phase is removed so effectively with a single rinse of distilled water, we do not believe that it contributes significantly to Sr/Ca variations observed in other cultures that have been rinsed at least twice with distilled water.

Table 2. *Field studies investigating controls of coccolith* Sr/Ca

region	material	fraction	reference	major result or test
Equatorial Pacific (140° W and 110° W)	surface sediments	polyspecific (less than 12 mm) and bulk	Stoll & Schrag (2000)	variation in Sr/Ca in coccolith (13%) and bulk carbonates (25%) covaries with productivity, $CaCO_3$ rain rate, alkenone-estimated growth rates
Lisbon upwelling transect	plankton	polyspecific (less than 63 mm)	Cachão *et al.* (2002)	other Sr-rich phase (Sr/Ca 14 mmol mol^{-1}—from acantharia $SrSO_3$?) overwhelms coccolith carbonate signal
Somalia upwelling transect	surface sediments	polyspecific and monospecific *C. leptoporus*, *G. oceanica*, *F. profunda*	new data	test relative amplitudes of Sr/Ca variation in different species: different relative contributions of temperature and productivity to coccolith Sr/Ca?
Equatorial Pacific (140° W)	surface sediments	monospecific *C. leptoporus*, *G. oceanica*, *F. profunda*	new data	test relative amplitudes of Sr/Ca variation in different species: different relative contributions of temperature and productivity to coccolith Sr/Ca?

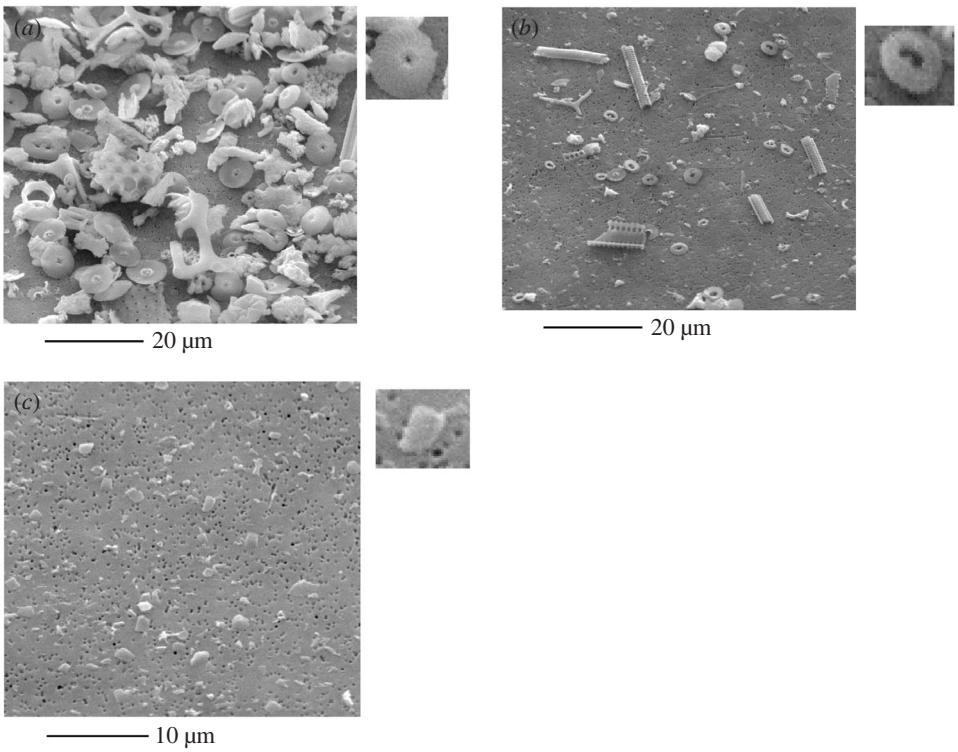

Figure 3. SEM images of different fractions separated from Equatorial Pacific sample MC58 (140° W, 0.41° N). (*a*) Fraction enriched in *C. leptoporus*. Other debris are fragments of diatom silica, which does not contribute significant Sr or Ca upon dissolution. (*b*) Fraction dominated by *G. oceanica*. (*c*) Fraction dominated by *F. profunda*. Separation techniques are described in Stoll & Ziveri (2002).

(*b*) *Field populations*

Until recently, the small size of coccoliths (3 to 10 μm) limited their separation from sediments, since they could not be readily 'picked' as could foraminifera. Previous studies of coccolith Sr/Ca in sediments have analysed polyspecific coccolith sediments (the less-than-12 μm fraction (Stoll & Schrag 2000)). New techniques now permit separation of fractions whose carbonate is highly dominated (greater than 70% and often greater than 90%) by a single coccolith species (Stoll & Ziveri 2002). These techniques are applied here for the first time to investigate species-specific changes in coccolith Sr/Ca across productivity gradients in two upwelling systems. We also describe results from a study of plankton samples collected across the upwelling front off the coast of Lisbon, Portugal. Recent field experiments focusing on coccolith Sr/Ca are summarized in table 2.

We analysed surface sediments collected along a transect perpendicular to the coast of Somalia by the Netherlands Indian Ocean Program (NIOP) in 1993, and samples from the Equatorial Pacific US Joint Global Ocean Flux Study transect at 140° W collected in 1993. In both transects, we separated samples of *C. leptoporus*, *G. oceanica* and *F. profunda* (figure 3). Samples were oxidized (method of Bairbakesh *et al.* (1999)), cleaned for minor element analysis (method of Apitz (1991)) and

separated, as described in Stoll & Ziveri (2002). Sr/Ca ratios were measured via inductively coupled argon plasma spectrometry in axial mode (thermo elemental model IRIS 1000 DUO at Middlebury College, Vermont, USA), as described for the culture samples in § 2 *a*.

We also analysed plankton samples in a transect through the Portuguese coastal upwelling region. Samples of living coccolithophorids were collected at several sites by filtering 20–40 l of sea water on cellulose nitrate filters. Particulates were removed from the filter, sieved to obtain the less-than-63 µm fraction, and rinsed in acetone (to dissolve any fragments of filter), ethanol and distilled water. Particulates were then dissolved in acetic acid/ammonium acetate buffer, as described in Apitz (1991).

3. New results

(*a*) *New culture results*

Table 3 shows the range of culture parameters and coccolith Sr/Ca for each species cultured, along with cross correlations.

Higher growth rates at higher irradiance lead to higher amounts of calcite per cell for both *G. oceanica* and *C. leptoporus* (figure 4*a*, *c*). For any given experiment, calcification per cell decreases with increasing cell density (figure 4*b*, *d*). In cultures of *H. carteri* and *S. pulchra*, no correlation was observed between higher growth rates and higher calcification/cell.

Sr/Ca ratios of rinsed *S. pulchra* of strains GK17 and GK7 varied by 7 and 8% over temperature ranges of 7 and 4 °C, respectively. Sr/Ca ratios are positively correlated with temperature, growth rate and calcification rate (figure 5*a*, *b*). There is no strong correlation with the cell density at harvest. The slope of Sr/Ca change with temperature is *ca.* 1% °C $^{-1}$ for GK17, but *ca.* 2% per ° C for GK7.

Sr/Ca ratios of rinsed *H. carteri* varied by nearly 10% over a temperature range of 10 °C and are positively correlated with temperature, growth rate and calcification rate (figure 5*c*, *d*). There is no strong correlation with the cell density at harvest. The slope of Sr/Ca change with temperature is *ca.* 1% °C $^{-1}$.

In *C. leptoporus*, the range of Sr/Ca with irradiance (10%) is comparable with that observed for *E. huxleyi*. However, only the samples with the very highest calcite production rates show a significant increase in Sr/Ca with calcification rate (figure 6). There is no significant trend of Sr/Ca with cell growth rate or pH. The tightest relationship is a direct one between Sr/Ca and calcification/cell, resulting both from lower calcite production rates at lower irradiance and from decreasing Sr/Ca and decreasing calcite/cell with increasing cell density for any given irradiance.

Experiments with *G. oceanica* at different irradiance levels also show a range of *ca.* 10% in Sr/Ca, similar to that observed with *E. huxleyi*. However, unlike the *E. huxleyi* experiments, the *G. oceanica* cultures show highest Sr/Ca ratios in samples with lowest growth and calcification rates rate (figure 6). This trend is not related to cell densities. There is a strong inverse correlation between Sr/Ca and pH, and the range in pH (7.60–7.99) is more than twice as great as in the *C. leptoporus* experiments (7.40–7.63). There is no significant relationship between calcification/cell and Sr/Ca.

The new cultures provide a much wider span of data comparing Sr/Ca variations both within and among species (figure 7*a*). Ranges of Sr/Ca ratios for different species overlap. Larger species with high organic carbon fixation rates and calcite production

Table 3. *Range of culture parameters and coccolith* Sr/Ca *for each species cultured, along with cross correlations*

	range		correlation							
	min.	max.	irrad.	growth rate	calcification (pg calc./cell)	calc. rate (pg calc./cell/day)	cell density at harvest	µg org. C/ml (at harvest)	µg carbonate/ml (at harvest)	pH
G. oceanica										
irradiance (µE m^{-2} s^{-1})	15	137								
growth rate (divs/day)	0.45	1.90	0.89							
calcification (pg calc./cell)	135	347	0.41	0.66						
calc. rate (pg calc./cell/day)	70	638	0.68	0.86	0.94					
cell density at harvest	34 000	452 500	0.25	0.08	−0.52	−0.36				
µg org. C/ml (at harvest)	1.41	18.75	0.25	0.08	−0.52	−0.36	1.00			
µg carbonate/ml (at harvest)	9.56	77.05	0.30	0.25	−0.30	−0.16	0.94	0.94		
pH	7.60	7.99	0.78	0.80	0.17	0.41	0.60	0.66	0.71	
Sr/Ca mmol mol^{-1}	2.34	2.68	−0.56	−0.82	−0.61	−0.68	−0.19	−0.19	−0.41	−0.76
C. leptoporus										
irradiance (µE m^{-2} s^{-1})	15	137								
growth rate (divs/day)	0.23	0.67	0.81							
calcification (pg calc./cell)	573	5 256	0.82	0.76						
calc. rate (pg calc./cell/day)	107	2 689	0.88	0.85	0.95					
cell density at harvest	3825	89 000	−0.41	−0.48	−0.65	−0.51				
µg org. C/ml (at harvest)	1.60	37.21	−0.41	−0.48	−0.65	−0.51	1.00			
µg carbonate/ml (at harvest)	9.68	102.50	0.28	0.43	−0.07	0.19	0.52	0.52		
pH	7.40	7.63	0.42	0.71	0.39	0.51	−0.42	−0.21	0.43	
Sr/Ca mmol mol^{-1}	2.60	2.91	0.41	−0.02	0.51	0.38	−0.46	−0.46	−0.60	−0.21

Table 3. (*Cont.*)

	range		correlation							
	min.	max.	temp. (°C)	growth rate	calcification (pg calc./cell)	calc. rate (pg calc./cell/day)	cell density at harvest	µg org. C/ml (at harvest)	µg carbonate/ml (at harvest)	
H. carteri										
temperature (°C)	13.8	23.5								
growth rate (divs/day)	0.09	0.40	0.86							
calcification (pg calc./cell)	1535	3347	0.47	0.42						
calc. rate (pg calc./cell/day)	198	1339	0.80	0.87	0.80					
cell density at harvest	5500	32 750	0.69	0.80	−0.10	0.46				
µg org. C/ml (at harvest)	13.8	82.3	0.69	0.80	−0.10	0.46	1.00			
µg carbonate/ml (at harvest)	12.1	82.0	0.88	0.94	0.50	0.88	0.80	0.80		
Sr/Ca mmol mol^{-1} (rinsed)	2.87	3.16	0.98	0.97	0.59	0.90	0.77	0.77	0.96	
S. pulchra										
temperature (°C)	13.8	23.5								
growth rate	0.08	0.22	0.64							
calcification (pg calc./cell)	830	1480	0.36	0.63						
calc. rate (pg calc./cell/day)	67	325	0.63	0.96	0.81					
cell density at harvest	2200	34 000	0.41	0.00	−0.49	−0.18				
µg org. C/ml (at harvest)	0.7	10.7	0.41	0.00	−0.49	−0.18	1.00			
µg carbonate/ml (at harvest)	7.2	32.1	0.42	0.17	−0.21	0.03	0.96	0.96		
Sr/Ca mmol/mol (rinsed)	2.37	2.80	0.88	0.86	0.52	0.78	−0.03	−0.03	0.15	

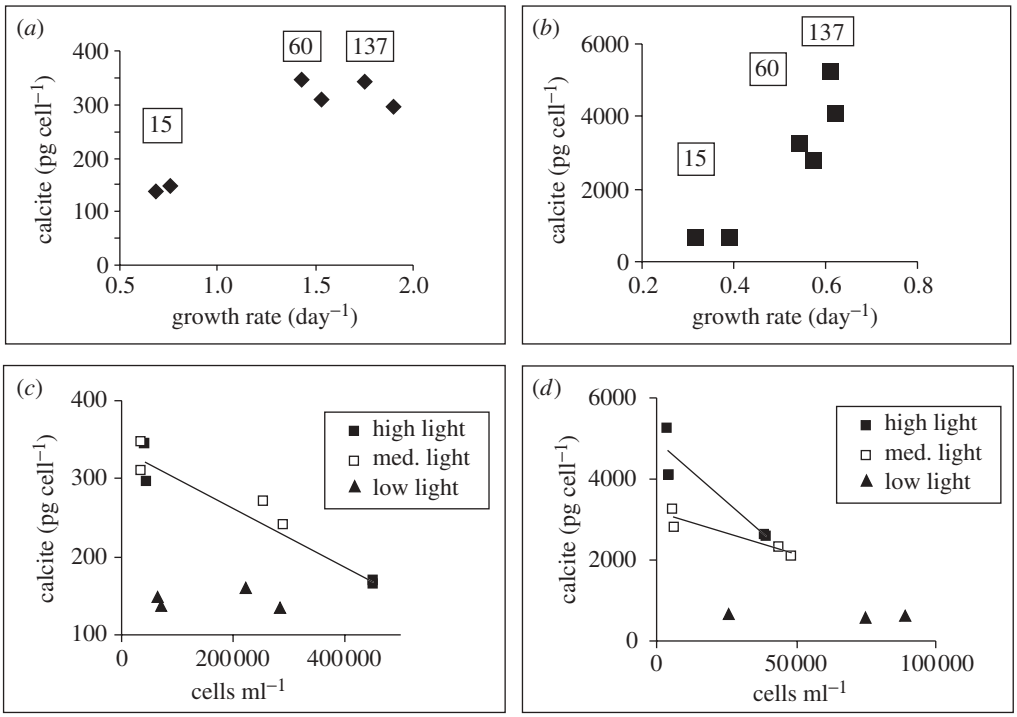

Figure 4. Calcification in light-limited batch cultures of *C. leptoporus* (a, b) and *G. oceanica* (c, d). Calcite per cell increases with increasing growth rate (a, c); boxes denote irradiance in μE m^{-2} s^{-1}. Decrease of calcification with increasing cell density (b, d) in response to dissolution of detached coccoliths.

rates like *C. leptoporus*, *H. carteri* and *C. pelagicus pelagicus* show the highest Sr/Ca ratios, but moderate ratios are also observed for species with much lower uptake rates in experiments like *G. oceanica* from the lowest light level (figure 7*b*).

(*b*) *Species-specific* Sr/Ca *responses to productivity across upwelling transects*

In the Somalia region the seasonal southwest monsoon causes extensive coastal upwelling from June to September, which enhances biological productivity in surface waters. During the upwelling event, living plankton concentrations show nearshore surface maxima and decrease with distance from the shore (Conan & Brummer 2000) and sea-surface temperatures may decrease from 29 to 22 °C.

Sr/Ca ratios in all fractions are higher in the near-shore region of highest upwelling intensity and productivity (indicated by high organic C contents and coccolith assemblages of the sediments; figure 8). Nearshore upwelling would tend to depress mean temperatures at the nearshore sites relative to offshore sites, so Sr/Ca variations are inversely correlated with temperature. The amplitude of Sr/Ca variations is much higher for *C. leptoporus* (greater than 50%) than for *G. oceanica* or *F. profunda* (both *ca.* 10%). All of these variations are much larger than the variation in Sr/Ca ratio of sea water, which is less than 2% throughout the ocean (De Villiers 1999). In the Equatorial Pacific region, persistent upwelling is strongest at the Equator, enhancing biological productivity there but depressing sea-surface temperatures

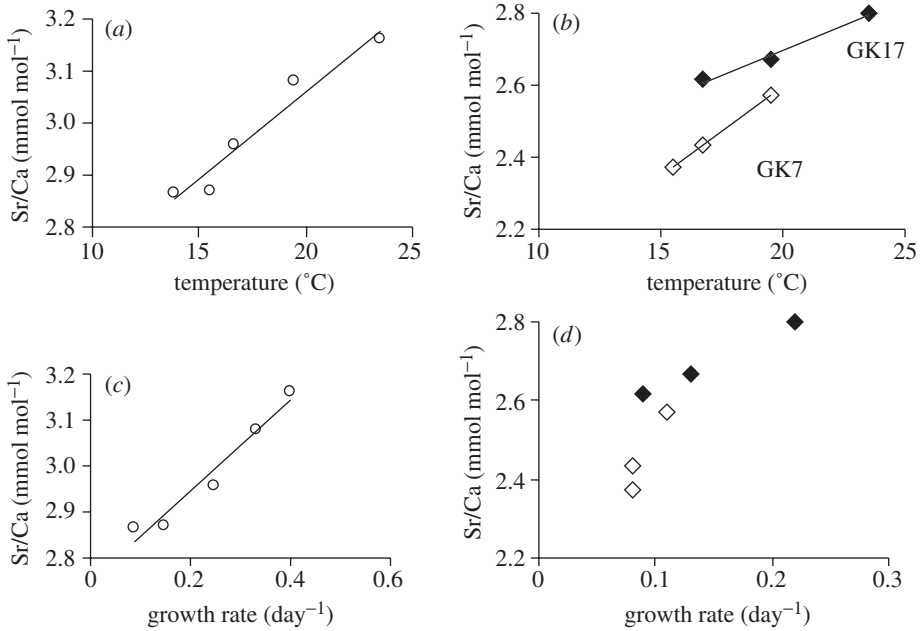

Figure 5. Relationships between Sr/Ca ratios and temperature (a, c) and growth rate (b, d) for *H. carteri* (a, b) and *S. pulchra* (c, d). Correlation coefficients are given in table 2. All data are from samples rinsed in distilled water.

by *ca.* 2 °C. Sr/Ca ratios in all fractions are higher in the equatorial region of highest upwelling intensity and productivity (figure 9). The amplitude of Sr/Ca variations is much higher for *C. leptoporus* (greater than 20%) than for *G. oceanica* or *F. profunda* (both 6% and 3%, respectively).

(*c*) *Plankton samples from the Portuguese upwelling transect*

Measured Sr/Ca in the fine particulate fractions were 13–14 mmol mol^{-1}, nearly twice the Sr/Ca ratio of sea water and five to seven times typical ratios for coccolith calcite (Stoll & Schrag 2000). This Sr/Ca ratio is not consistent with Sr partitioning constants for any biogenic carbonates. One plausible explanation, detailed in Cachão *et al.* (2002), is that there is some acantharian-derived celestite ($SrSO_4$) in these samples. Acantharia are widely distributed in the upper water column and are known to produce abundant celestite granules of 1–3 μm, and in shallow (400 m) sediment traps in the Atlantic Ocean (32° N, 64° W), acantharian celestite may contribute up to 67% of less-than-63 μm particles (Bernstein *et al.* 1993). Consequently, it is not possible to infer anything about Sr/Ca ratios of coccoliths from these samples.

4. Discussion

(*a*) *Consistency of culture results*

Culture experiments, both batch and continuous, give consistent results for a number of species. Higher calcification per cell at higher growth rates observed in light-limited cultures of *G. oceanica* and *C. leptoporus* here, as well as in *E. huxleyi* cultures

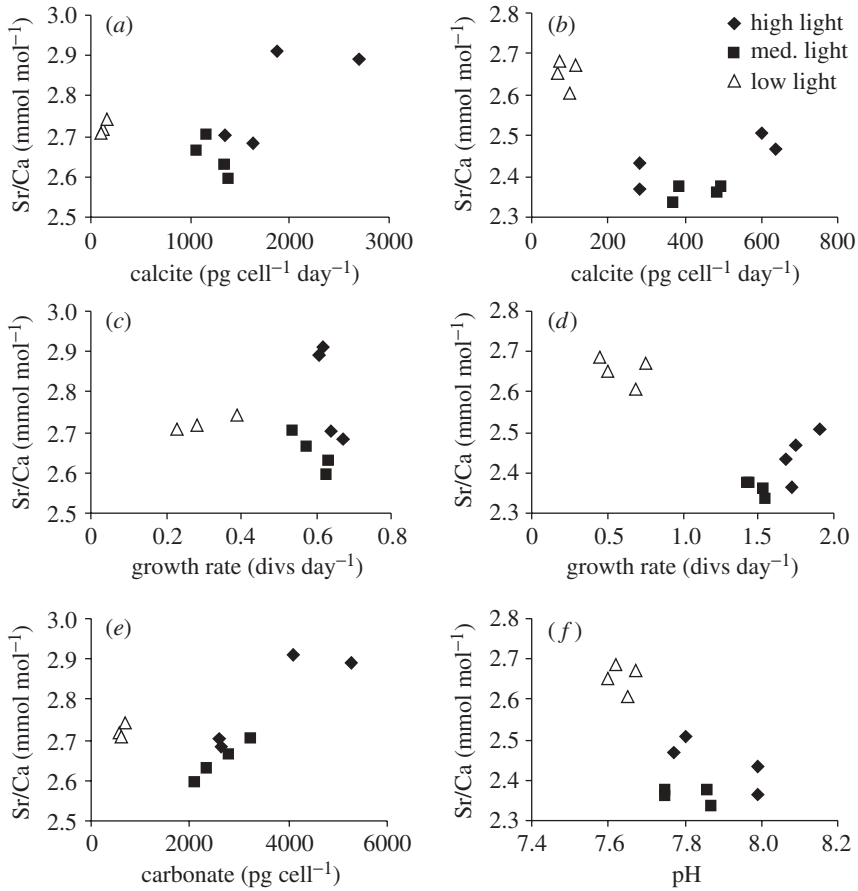

Figure 6. Sr/Ca ratios for *C. leptoporus* (*a–c*) and *G. oceanica* (*d–f*) versus calcification rate (*a*, *d*), growth rate (*b*, *e*), calcification per cell (*c*) and culture pH (*f*). Correlation coefficients are given in table 2. All data are from samples rinsed in distilled water.

(Paasche 1999; Stoll *et al*. 2002*b*), may suggest that active uptake and calcification become increasingly important at higher growth rates. Decreases in calcification per cell with increasing cell density appears to be common in batch cultures of numerous species (Stoll *et al*. 2002*b*), as well as in ocean bloom environments (van Bleijswijk 1996), and are likely due to increased dissolution of coccoliths (see, for example, van Bleijswijk 1996). In early log phase growth, most coccoliths are attached to cells, but as cultures enter late log phase, the number of detached coccoliths can exceed attached coccoliths by a factor of two to three (Balch *et al*. 1993), and these detached coccoliths may be more prone to dissolution. In batch cultures, this dissolution process is likely accentuated by the progressive drawdown of media Ca and alkalinity as cell densities increase and is most pronounced in the highest calcifying species and strains, where alkalinity drawdown is greatest (Stoll *et al*. 2002*a*).

Correlation between calcification per cell and growth rate in cultures of *H. carteri* and *S. pulchra* may be obscured by the sampling bias of denser cultures in faster growing experiments, since in these denser cultures the amount of calcite/cell is reduced.

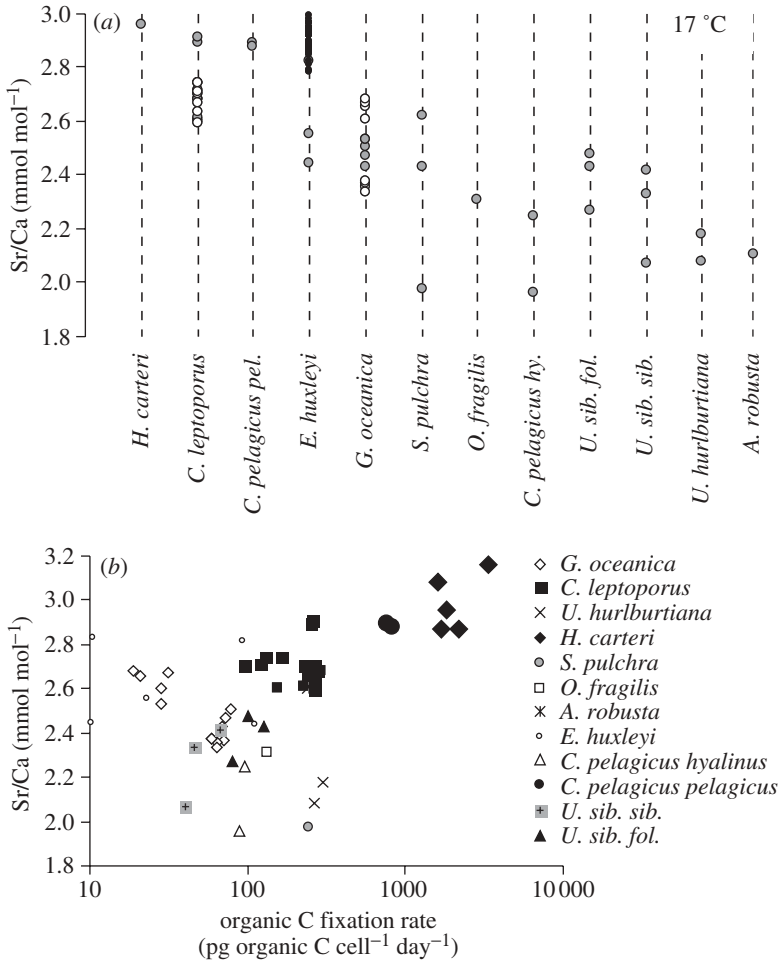

Figure 7. (*a*) Sr/Ca ratios in all cultures at 17 °C by species. Solid symbols indicate results from light-saturated conditions, open symbols denote light-limited conditions. Smaller symbols for *E. huxleyi* indicate samples from continuous culture in a turbidostat system (Stoll *et al*. 2002*b*). (These turbidostat samples were measured using a different standard series not yet cross-checked with standards used in other culture experiments.) (*b*) Sr/Ca ratios in all batch cultures versus organic carbon fixation rate estimated from cell biovolume and growth rates.

New culture experiments with *S. pulchra*, *H. carteri* and *A. robusta* confirm previous observations with *E. huxleyi* and *G. oceanica*, showing higher Sr/Ca ratios at higher temperatures (figure 8). Sr/Ca increases between 1 and 2% °C $^{-1}$. While part of this increase in Sr/Ca may be due to higher growth rates at higher temperatures, for both *E. huxleyi* and *G. oceanica*, the trend of increasing Sr/Ca with increasing temperature is much steeper than observed over a similar range in growth rate induced by light limitation (Stoll *et al*. 2002*a*, *b*). Consequently, temperature appears to exert an additional influence on coccolith Sr/Ca. The slope of this relationship of Sr/Ca with temperature is similar to published relationships in planktonic foraminifera (Lea *et al*. 1999) and abiogenic calcites (Malone & Baker 1999).

Where light is used to control growth rates, culture studies with several species

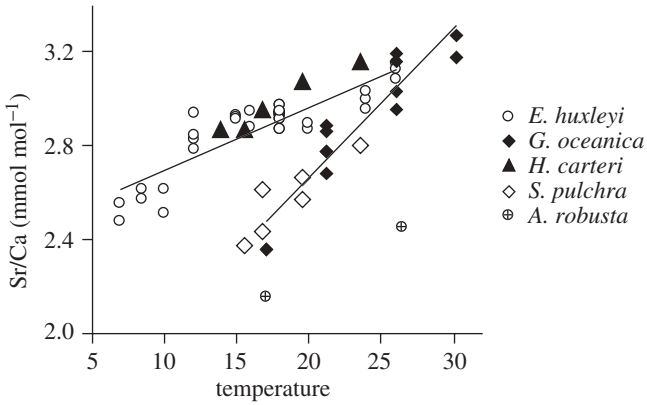

Figure 8. Sr/Ca ratios in monospecific samples separated from surface sediments in the Somali upwelling region (*a*) compared with indicators of productivity (*b*). Per cent organic carbon from Ivanova *et al*. (1999). Per cent oligotrophic coccoliths from P. Ziveri (unpublished data).

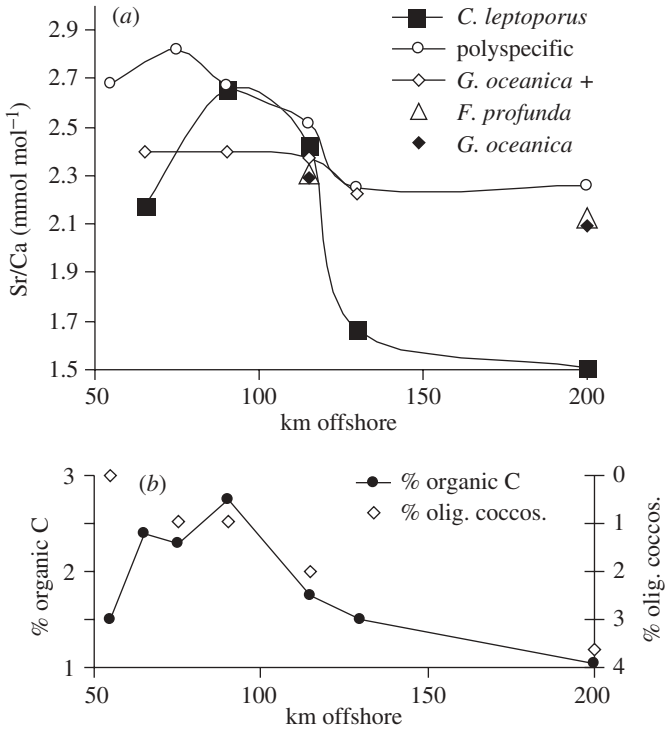

Figure 9. Sr/Ca in monospecific samples separated from surface sediments in (*a*) the Equatorial Pacific upwelling region (140° W) compared with (*c*) indicators of productivity. Alkenone estimated growth rates from Bidigare *et al*. (1997). Primary productivity in mg C m^{-2} d^{-1} from Chavez *et al*. (1990), Barber *et al*. (1991) and Chavez *et al*. (1998). fCO_2 data from Takahashi *et al*. (1994).

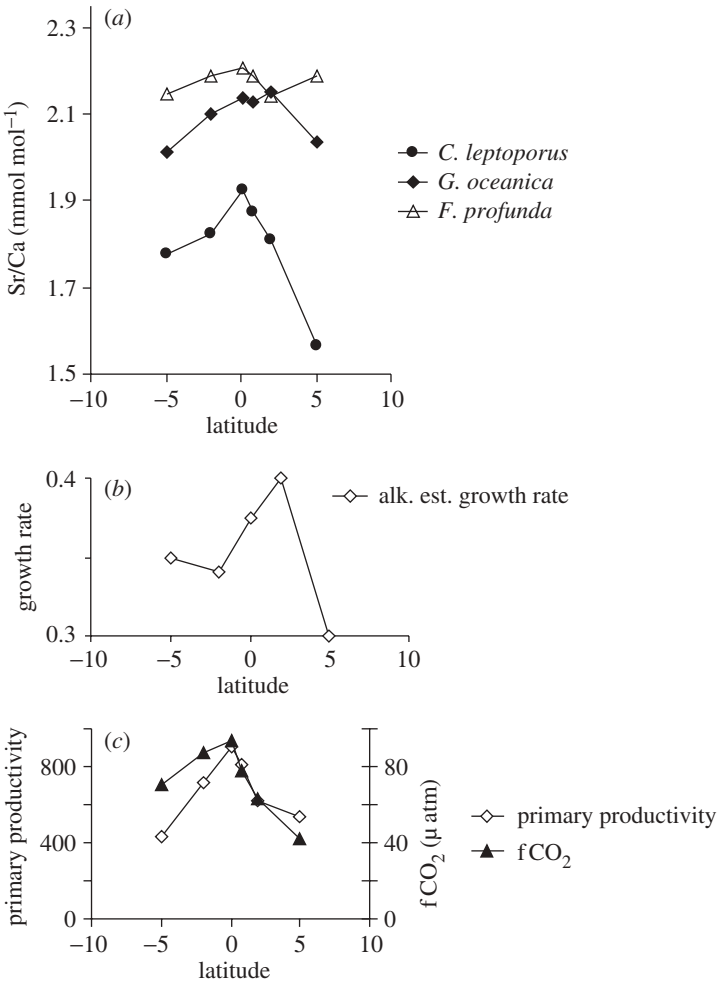

Figure 10. Sr/Ca versus temperature in a range of batch and continuous culture experiments. Data for *E. huxleyi* from Stoll *et al*. (2002*b*). Data for *G. oceanica* from Stoll *et al*. (2002*a*). Linear best fits are shown for *E. huxleyi* ($y = 0.0272x + 2.4238$; $r^2 = 0.78$) and *G. oceanica* ($y = 0.0636x + 1.3944$; $r^2 = 0.88$).

(*E. huxleyi*, *G. oceanica* and *C. leptoporus*) consistently reveal a relatively small range in coccolith Sr/Ca, typically around 10%, over wide ranges in growth rate (Stoll *et al*. 2002*b*). In light-limited experiments, Sr/Ca ratios are not always related to growth rate, as seen most notably in the *G. oceanica* results here. This indicates that some factor other than growth rate (and temperature) must ultimately be influencing the Sr/Ca ratio in culture experiments.

In the case of *G. oceanica*, there was a strong correlation between pH and coccolith Sr/Ca. However, pH in batch cultures is a chicken-and-egg problem: pH influences the speciation of carbon in the media and hence the C available for algal growth, but the progressive increase in culture cell density (removal of dissolved C and alkalinity) also alters pH in ways that may be different depending on the ratio of organic to calcite carbon fixed by the coccolithophorids. In these *G. oceanica* cultures, external factors

seem to be responsible for the pH variations among the different light experiments, since the lower calcite/organic carbon ratio of the coccolithophorids in the low light would tend to increase pH faster than in the more calcified higher light cultures, yet observed pH values are lower than in more calcified cultures. However, it is unclear how the strong pH differences in the *G. oceanica* media may affect Sr/Ca ratios in coccoliths.

Simple relationships between Sr/Ca and calcite-production rate, organic-C-fixation rate or cell size observed in previous studies (Stoll *et al*. 2002*a*) are not apparent in a broader range of conditions, although the largest species with highest organic C fixation rates show the highest Sr/Ca ratios in culture and the large *H. carteri* also showed the highest Sr/Ca ratios in surface sediments from the Atlantic (Stoll & Ziveri 2002). Consequently, a simple calcification rate or organic-C-fixation rate control cannot be invoked to explain both intraspecific and interspecific correlations between Sr/Ca and calcification rate observed in cultures. Furthermore, if the relationships between Sr/Ca and calcification among different species are not the same as those relationships in species, interpretation of polyspecific Sr/Ca variations in sediments will be more difficult.

(*b*) *Consistency of field and culture studies*

The amplitude of the Sr/Ca variations in *C. leptoporus* (23–50%) is much larger than observed for light-limited growth rates in culture (10%). It seems unlikely that there would be a larger range of growth rates for *C. leptoporus* across the upwelling transects than that observed in culture. Instead, it appears that the productivity variations across the upwelling transects induce a steeper response in coccolith Sr/Ca. Across the upwelling transect, productivity variations are likely driven by micronutrient availability and/or CO_2 variations. Consequently, variations in growth rates appear not to be the ultimate control over coccolith Sr/Ca ratios, although the factor controlling coccolith Sr/Ca is still highly correlated with productivity in the ocean, at least across upwelling zones. Also, experiments with clonal cultures may not capture the full range of responses that would be observed in genetically diverse marine populations. The large response of *C. leptoporus* in the field compared to cultures may also reflect different responses in the intermediate morphotype (sub)species (grown in culture) versus the large morphotype (found in Somalia upwelling region), as well as more subtle shifts in (sub)species across these transects from the areas of maximum upwelling to the intermediate type in more oligotrophic conditions (see, for example, Knappertsbusch *et al*. 1997; Renaud & Klaas 2002; Geisen *et al*. 2002). Different adaptations in these (sub)species could influence coccolith Sr/Ca ratios.

The much higher amplitude Sr/Ca variations of *C. leptoporus* relative to other species also contrasts with culture studies where light-induced Sr/Ca variations were similar in magnitude to those of *E. huxleyi* and *G. oceanica*. It is unclear whether the reduced responses of coccolith Sr/Ca in some species reflect actually lower amplitude productivity responses to upwelling, or whether they reflect comparable productivity responses but a lower slope of the relationship between productivity and Sr/Ca variations. Larger species like *C. leptoporus* may have a more dynamic response if their cell size (and lower surface area/volume ratio) restricts diffusive uptake of limiting nutrients (CO_2 or micronutrients) in more oligotrophic regions, so the greater availability of limiting nutrients in upwelling zones represents a greater relative stimulus than for smaller cells of other species. Field studies combining species-specific

measurements in growth rates (via diel DNA content changes) and coccolith Sr/Ca measurements might provide the most accurate answer to this question. If there were a real variation in the response of these organisms to productivity changes that was accurately recorded by coccolith Sr/Ca, it would provide an interesting perspective on past and present variations in ecological structure of coccolithophorid communities.

Temperature effects may be consistent in culture and field studies. Although coccolith Sr/Ca ratios across the field transects are inversely related to coccolith Sr/Ca, whereas cultures show a direct increase in Sr/Ca of 1–2% per °C, a small temperature effect could easily be masked by the large productivity response in the field transect.

(*c*) *Reviewing* Sr/Ca *in polyspecific and bulk carbonate*

Bulk carbonate in modern deep-sea sites is composed of coccoliths, foraminifera and, in shallower sites, aragonitic pteropods. Because coccoliths have Sr/Ca ratios nearly double those of most planktonic foraminifera, the Sr/Ca ratio of bulk sediments is sensitive to changes in the proportion of foraminiferal to coccolith carbonate, as well as changes in the Sr/Ca ratio of either coccoliths or foraminifera (Stoll & Schrag 2000). In transects across the Equatorial Pacific, the relative contribution of Sr/Ca from coccoliths was positively correlated with the Sr/Ca ratio of the coccoliths (Stoll & Schrag 2000). This may reflect that at higher productivities, coccoliths have higher Sr/Ca ratios and also contribute more carbonate to the sediments. This relationship may make it possible to infer general palaeoproductivity trends from bulk Sr/Ca variations in older sediments where it is not possible to separate coccoliths from other components, especially in sediments dominated by coccolith carbonate. However, in sediments with abundant foraminiferal carbonate, dissolution changes may bias the foraminifera/coccolith ratio, altering the bulk carbonate Sr/Ca ratio with no changes in productivity of overlying surface waters. Furthermore, the large heterogeneities of Sr/Ca among modern species suggest that bulk carbonate Sr/Ca may not be adequate for high-resolution work in the Quaternary.

5. Mechanisms for coccolith Sr/Ca variations

Coccoliths are produced intracellularly and it is unclear to what extent their chemistry is controlled by chemical kinetic versus biological factors. Coccolith Sr/Ca may be controlled by kinetic or thermodynamic effects on the partitioning of Sr and Ca into the forming crystal lattice, thermodynamic effects on the enrichment of Sr on the surface of the growing crystal, or biological controls over the Sr/Ca ratio of the intracellular fluid from which the calcite precipitates.

Previous studies have suggested that kinetic effects on Sr partitioning might control Sr/Ca variations in biogenic carbonates like coccoliths and foraminifera (Stoll & Schrag 2000; Lea *et al*. 1999), since experimentally precipitated abiogenic experiments show strong kinetic effects on Sr (see, for example, Lorens 1981; Tesoriero & Pankow 1996). Stoll *et al*. (2002*b*) tested this prevailing assumption by comparing culture results from *E. huxleyi* with numerical models of kinetic effects resulting from surface enrichment during crystal growth, such as those developed by Watson & Liang (1995) and Watson (1996). This model–data comparison showed that surface enrichment effects can produce only small variations in Sr/Ca ratios over the

range of crystal growth rates inferred for coccolith calcite. For example, the small Sr variations among *E. huxleyi* coccoliths at different calcification rates at constant temperature may result from kinetic effects during crystal growth. However, effects unrelated to the kinetics of crystal growth (such as changes in the surface enrichment of Sr on the growing surface of the crystal, changes in equilibrium lattice Sr partitioning or changes in the Sr/Ca ratio of the calcifying fluid) must be responsible for the larger Sr/Ca variations observed in other culture and field studies.

In the case of temperature-induced variation, the constancy of the temperature–Sr/Ca slope in coccoliths and other biogenic and abiogenic calcites suggests that it arises from thermodynamic effects on Sr partitioning. While Sr does not form a stable solid solution series in the calcite mineral structure, there still may be temperature-related thermodynamic controls of surface enrichment during crystal growth.

Differing enrichment of Sr on growing crystal surfaces appears to be a lesser control of coccolith Sr/Ca, at least at constant temperatures. Although different Sr partitioning has been reported on different crystal growth faces of calcite (Paquette & Reeder 1995), Sr/Ca ratios among different species do not appear to be strongly influenced by the orientation of the *c*-axis in the crystal units. In *E. huxleyi* and *G. oceanica*, nearly all of the calcite is made of crystal units with sub-radially oriented *c*-axes ('R'), whereas in other species investigated here, the calcite is roughly equally from crystal units with sub-radial and sub-vertical *c*-axes ('V/R' (Young *et al*. 1999)). However, there are no clear separations in Sr/Ca ratios between these two 'R' and all the other 'V/R' species on figure 8. This suggests that variations in the Sr/Ca ratio of the calcifying fluid may be a more important control over coccolith Sr/Ca.

Models of surface enrichment effects indicated that Sr/Ca ratios in the calcifying fluid may be higher than those of sea water. Higher calcite precipitation rates in biogenic calcites are not sufficient to cause the higher Sr/Ca ratios observed in biogenic calcites relative to abiogenic calcites, as has been suggested by previous workers (Carpenter & Lohmann 1992). The high Sr/Ca ratios of coccoliths require that either the surface enrichment for coccolith calcite is nearly twice as great as in abiogenic precipitation experiments or the Sr/Ca ratio of the calcifying fluid is nearly twice as great as that of sea water.

The Sr/Ca of the fluid is likely to be regulated by ion transport and membrane permeability. Although early culture work with *E. huxleyi* indicated that enrichment of the media in Sr promoted attachment of coccoliths to the cells (Sikes & Wilbur 1980), no studies have demonstrated a specific biological role for Sr. Consequently, Sr is likely to be incorporated to the calcifying fluid through non-specific channels. Calcifying fluids with Sr/Ca ratios higher than sea water would not be surprising, since in other Ca-permeable channels in plants, Sr (and Ba) often permeate more readily than Ca (C. Brownlee 2000, personal communication).

Nonetheless, we have no data revealing how changes in the Sr/Ca ratio of the calcifying fluid are linked with variations in the productivity of the cell. Rickaby *et al*. (2002) suggest that there may be a rate-dependent discrimination between the biological transport of Sr^{2+} and Ca^{2+} associated with passive transport through ion channels, or active pumping via carrier proteins. This model invokes selectivity against Sr in uptake. In exponentially growing cells, bicarbonate enters via a Ca^{2+}/HCO_3^- symport (Brownlee *et al*. 1994). If HCO_3^- pumping is enhanced at faster growth rates as an additional source of C for photosynthesis (as implied by

our data showing higher calcification per cell at higher growth rates), then increased HCO_3^- would entail an increased rate of supply of Ca through the ion pumps. If there is selectivity against Sr in this symport, which is reduced at higher pumping rates, the Sr/Ca of the calcifying fluid could increase. Given that coccolith Sr/Ca does not increase with growth and calcification rate under all cultures, and the magnitude of culture and field Sr/Ca variations is very different, the actual mechanism of Sr/Ca variations in coccoliths must be more complex. While the carrier protein or ion-pumping mechanisms provide a useful conceptual framework, further study is needed to understand the mechanisms of Sr variation in coccoliths and their relationships with other biochemical cycles in the cell.

6. Other geochemical work on coccoliths

Since there appear to be multiple factors influencing coccolith Sr/Ca, including temperature and growth rate, it may be useful to examine other proxies from coccolith carbonate. If temperature and growth rate have a different relative influence on other trace metals or stable isotopes in coccoliths, combined geochemical data may give us more confidence in interpreting coccolith palaeoclimate signals, as well as constraining biological mechanisms for Sr/Ca variations.

(*a*) Mg/Ca

Mg incorporation in calcite is strongly dependent on temperature, so Mg/Ca of biogenic calcites are increasingly being used for palaeothermometry. Measurement of coccolith Mg/Ca is complicated by the low Mg/Ca ratios of coccolith carbonate (0.1–0.2 mmol mol^{-1}, one to two orders of magnitude lower than in foraminiferal calcite) compared to high Mg contents in algal organic matter. Typical *E. huxleyi* samples from culture experiments contain 5–25 $\times 10^{-14}$ g Mg $cell^{-1}$ in organic fractions, 100–500 times higher than that of the $CaCO_3$, which contains only 5×10^{-16} g Mg $cell^{-1}$ (Y. Rosenthal, unpublished data).

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 (Stoll *et al*. 2001). Because coccoliths are much smaller and have a much lower Mg content (compared to foraminifera), the potential advantages of a coccolith Mg/Ca palaeotemperature proxy may be outweighed by the greater complexity of cleaning issues. Alkenone undersaturation indices (Uk^{37}) may provide the most reliable index of temperatures at which coccolithophorids are growing to account for this effect on coccolith Sr/Ca.

(*b*) *Stable isotopes*

Under equilibrium conditions, the $\delta^{18}O$ of marine carbonates depends on both temperature and the $\delta^{18}O$ composition of sea water, while the $\delta^{13}C$ is controlled by the $\delta^{13}C$ of dissolved inorganic carbon (DIC) and temperature. Cultures of several species of coccolithophores revealed a large range of stable isotopic compositions, indicating important non-equilibrium effects in the stable isotope fractionation of coccolith calcite (Dudley *et al*. 1986). Dudley *et al*. (1986) hypothesized that different growth or calcification rates could influence oxygen isotope partitioning, but did not present quantitative growth rate data.

Recent results from cultures of several species of coccolithophorids show that in light- and nutrient-replete cultures the non-equilibrium effects in $\delta^{18}O$ correlate highly with cell division rates across a range of growth rates rate (Ziveri *et al.* 2000, 2002). Systematic relationships were found between the carbon and oxygen isotopic composition of the coccolith calcite and the surface area/volume ratio of the cells, which sets the diffusive flux of CO_2 available to the cell. These data suggest that stable isotope fractionation in coccoliths is related to the dynamics of carbon uptake. Consequently, the non-equilibrium effects in coccolith stable isotopes may constrain the roles of diffusive and active carbon uptake at different growth rates in different species in models of cellular carbon acquisition (Keller & Morel 1999). Coupled stable isotope and Sr/Ca data from culture coccoliths may therefore help constrain the relationships between Sr/Ca variations and other biochemical cycles in the cell.

7. Conclusions

Laboratory cultures of coccolithophorids indicate that temperature exerts an additional influence on coccolith Sr/Ca, beyond the effect of temperature on growth rate. Increases in coccolith Sr/Ca with temperature of 1–2% $^{\circ}C^{-1}$ are observed in all species and probably represent a ubiquitous effect that must be considered in all palaeoceanographic studies.

Light-limited cultures consistently show a small range of variation in coccolith Sr/Ca across a large range in cell growth rates. In some experiments, coccolith Sr/Ca was not directly related to growth or calcification rate. However, field data from two upwelling transects indicate a robust relationship between coccolith Sr/Ca and coccolithophorid productivity. Consequently, it is still possible that coccolith Sr/Ca reliably indexes coccolithophorid productivity in the ocean, which may be driven by nutrient and C limitation (rather than light limitation). Ideally, this Sr/Ca–productivity relationship should be confirmed with a study of surface sediments on a global scale. A study over a larger geographic distribution would also show the response of Sr/Ca over a wider range in temperatures. New techniques in separating monospecific fractions from sediments should facilitate this type of comparison in future studies. However, because the species assemblages vary from one region to another, it will be more difficult to separate a single species from a wide geographic area to compare coccolith Sr/Ca on a larger scale.

More generally, we would note that our strategy for evaluating the Sr/Ca proxy has been to integrate data from a range of approaches, including culture experiments, to attempt both to test the proxy and to develop a mechanistic model of the underlying process. This approach has revealed many complications and it would be true to say that the situation is currently somewhat unclear. However, we strongly feel that this is a necessary compliment to extensive geological application of geochemical proxies based on simplistic models. Most potential geochemical proxies are probably under complex physiological and thermodynamic control. Deepening our understanding of such controls should be as high a priority as broadening the range of palaeoceanographic proxies used.

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