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Earth and Planetary Science Letters 210 (2003) 137–149

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Stable isotope 'vital effects' in coccolith calcite

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Received 9 September 2002; received in revised form 24 February 2003; accepted 26 February 2003

Abstract

Uncertainties about the origin of the many disequilibrium or 'vital effects' in a variety of calcifying organisms, and whether these effects are constant or variable, have hampered paleoceanographic application of carbon and oxygen isotopic ratios. Unraveling the source of these effects will improve paleoceanographic applications and may provide new information on changes in cell physiology and ecology. Culture of eight species of coccolithophorids, a dominant marine phytoplankton group, reveals a 5‰ array of disequilibrium or 'vital effects' in both the carbon and oxygen isotopic composition of coccolith calcite. In moderate light and nutrient-replete cultures, oxygen isotopic fractionation and carbon isotopic fractionation correlates directly with cell division rates and correlates inversely with cell size across a range of species. However, when growth rates of a single species are increased or decreased by higher or lower light levels, $\varepsilon^{18}O$ is relatively invariant. Likewise, growth rate variations as a function of temperature do not influence coccolith $\varepsilon^{18}O$; the slope of the $\varepsilon^{18}O$ vs. temperature relation in cultures of both *Gephyrocapsa oceanica* and *Helicosphaera carteri* is the same as for abiogenic carbonates. This suggests a constant, species-specific isotopic fractionation which does not vary with cell physiology. The constancy of vital effects suggests that coccolith stable isotopes will provide reliable phase for paleoceanographic reconstruction of temperature and seawater chemistry, as long as monospecific fractions are analyzed or changes in nannofossil assemblages are accounted for with species-specific correction factors. We suspect that the cell size, and its constraints on the rate of CO_2 diffusion relative to C fixation, may be the first order influence on coccolith stable isotope vital effects. A quantitative model of this process may provide important constraints on mechanisms of carbon acquisition of coccolithophorids in both modern and extinct species.

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doi:10.1016/S0012-821X(03)00101-8

Keywords: coccolithophorids; coccoliths; stable isotopes; $\delta^{18}O$; $\delta^{13}C$; vital effect

1. Introduction

The oxygen isotopic ratio of marine biogenic carbonates is widely used to reconstruct the temperature of ancient oceans and to trace changes in the oxygen isotope ratio of seawater which varies with global ice volume. The carbon isotope ratio is used to reconstruct variations in the carbon isotopic composition of dissolved inorganic carbon in the ocean, which is controlled by biological productivity through the removal of isotopically light carbon in organic matter. However, the carbon and oxygen isotopic composition of many biogenic carbonates differs from that expected for equilibrium precipitation in seawater, an offset referred to as a 'vital effect' or biological fractionation. Most paleoceanographic studies have circumvented this problem by isolating carbonate from a single species and assuming that the vital effect in a given species is constant and does not vary through time. The relative changes in isotopic values can then be interpreted as changes in temperature or in the isotopic composition of seawater.

To date, this approach is mainly applied on foraminifera but not on coccoliths, tiny plates produced by coccolithophorid algae, which are often the most dominant carbonate contributors to pelagic sediments. Coccoliths may be the most important carbonate phase for geochemical analysis in sediments where foraminifera are less common and/or core material is limited, such as in subpolar regions and for Early Cenozoic and Mesozoic sediments. However, coccoliths are too small (2–12 μm) to be 'picked' individually under a microscope as is routinely done for foraminifera, making single species isolations difficult. Cultures of coccolithophorids show an especially wide range of vital effects with a range of nearly 5‰ in oxygen isotopic composition of coccoliths from different species grown under similar culture conditions [1–3]. Consequently, changes in the relative carbonate contribution of different species in sediments may cause significant changes in the isotopic ratios of the assemblage, partially masking signals of changing temperature and seawater isotopic composition. If the magnitude of the vital effects of different species can be determined from culture experiments, it may be possible to correct for changing nannofossil assemblages (e.g. [4]). However, this approach cannot be extended to extinct nannofossil species in older sediments. Furthermore, since the origin of the large vital effects in coccoliths is not yet understood, it may not be reliable to assume that these vital effects are constant. Recent work in corals [5] and foraminifera [6] has shown that stable isotopic vital effects may vary with environmental conditions. Far from being a drawback, these vital effects themselves are now serving as valuable new paleoceanographic indicators.

The full potential of coccolith stable isotopes in paleoceanographic studies can be reached when we can resolve the constancy and origin of the stable isotopic vital effects in coccolith carbonate. Through culture of large numbers of coccolithophorid species under a range of experimental conditions in this study, we address the following:

- Are the offsets or 'vital effects' constant for a given species, or do they vary with physiological factors like growth or calcification rate?
- What can the vital effects themselves tell us about the ecology or physiology of the coccolithophorid algae?

Our study is based on three sets of experiments. In the first set of experiments, we grew eight species of coccolithophorids under identical environmental conditions (nutrients, temperature, light) to investigate the array of interspecific vital effects and their relationship with the physiological responses of the different species. These experiments document a strong relationship between oxygen isotope fractionation and cell growth rates, which if operative at different growth rates in a single species could imply that vital effects are not constant for a given species. To test the effect of varying growth rates on vital effects in each species, we completed a second set of experiments

Table 1
Coccolithophorid species and strains cultured in this study

Strain	Species	Isolated from	Date isolated
JS1 A	*Gephyrocapsa oceanica*	Southern Mediterranean	5/98
A4725	*Gephyrocapsa oceanica*	Gulf Stream, USA	4/97
A4727	*Gephyrocapsa oceanica*	Gulf Stream, USA	4/97
ARC3	*Gephyrocapsa oceanica*	Atlantic Shelf, France	6/98
ESP186	*Gephyrocapsa oceanica*	Western Mediterranean, Spain	2/98
JS8	*Gephyrocapsa oceanica*	Southern Mediterranean	5/98
PC71	*Gephyrocapsa oceanica*	Atlantic Shelf, Portugal	7/98
MS1	*Emiliania huxleyi*	North Sea	98
TQ26	*Emiliania huxleyi*	Tasman Sea (New Zealand)	10/98
KL2	*Coccolithus* ph. *pelagicus*	Atlantic Shelf, France	12/98
LK1	*Coccolithus pelagicus* ph. *hyalinus*	Atlantic Shelf, France	11/98
PC11M3 B	*Calcidiscus leptoporus*	Atlantic Shelf, Portugal	7/98
ASM 36	*Umbilicosphaera sibogae sibogae*	Western Mediterranean (Alboran Sea)	10/99
ESP6 M1	*Umbilicosphaera sibogae foliosa*	Western Mediterranean, Spain	4/99
GK17	*Syracosphaera pulchra*	Western Mediterranean, Spain	5/00
AS641	*Olithothus fragilis*	Western Mediterranean (Alboran Sea)	10/99
NS8-04	*Helicosphaera carteri*	South Atlantic (South Africa)	9/00
NS10-8	*Helicosphaera carteri*	South Atlantic (South Africa)	9/00
ASM24	*Algirosphaera robusta*	Western Mediterranean (Alboran Sea)	10/99

where different light levels were used to change growth rates of *Calcidiscus leptoporus* and *Gephyrocapsa oceanica*. We also completed a third set of experiments, growing *G. oceanica* and *Helicosphaera carteri* at different temperatures, to assess whether growth rate variations with temperature affect the slope of the paleotemperature equation for oxygen isotopes.

These experiments are the first in which a wide array of culture parameters were determined, as well as the first measurements of vital effects on $\delta^{13}C$ of coccoliths grown in culture.

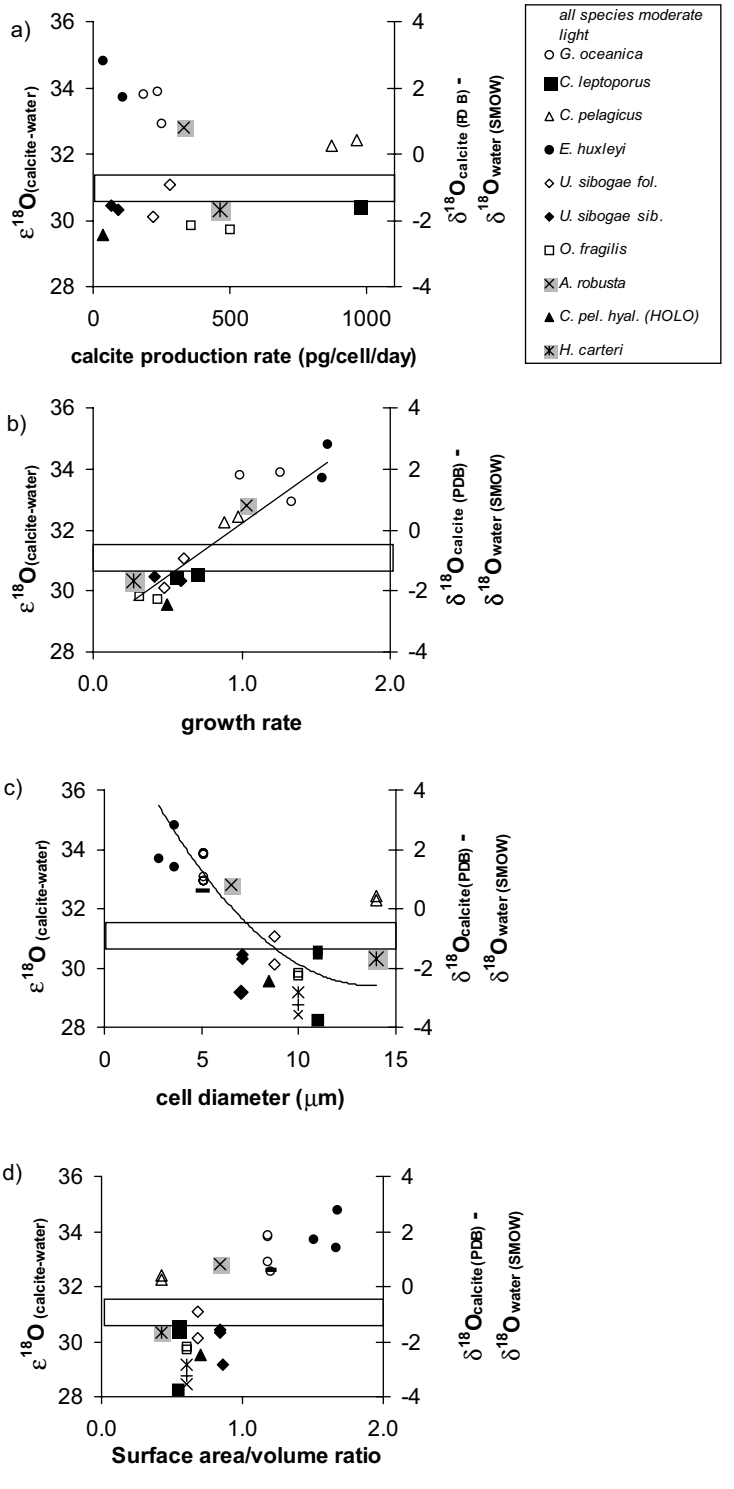
2. Methods

2.1. Culture experiments

Batch cultures of 300 ml were grown in filter-sterilized coastal seawater enriched in nutrients, vitamins, and trace metals to standards for K/2 medium [7]. Species cultured, their strain and origin are given in Table 1. Cultures were grown on a 14:10 light:dark cycle. In all experiments, cultures were acclimatized to the experimental conditions (growth medium, temperature and light) for at least 2 weeks (>10 cell divisions) prior to inoculation. Culture media and coccoliths were sampled several times in log phase at low cell densities before significant alteration of medium alkalinity and pH. We report carbon isotopic fractionation only from samples collected during early log phase. Log phase growth rates for each culture experiment are calculated from frequent cell density measurements with a hemocytometer.

In one experiment to compare vital effects across a range of species, eight species (Table 1) were grown at a constant temperature of 17°C and moderate light conditions of 60 (± 10) μE m^{-2} s^{-1}. In a second experiment, designed to assess the constancy of vital effects in a given species under varying conditions, cultures of *C. leptoporus* and *G. oceanica* were grown at constant temperature but three irradiance levels: 15 (± 5), 60 (± 10), and 137 (± 13) μE m^{-2} s^{-1}. For these experiments, two replicates of a single strain were cultured. In a final experiment, six strains of *G. oceanica* were cultured at temperatures ranging from 17 to 30°C, and two strains of *H. carteri* were cultured at temperatures from 17 to 23°C.

Ten to thirty milliliter of culture was centrifuged down to concentrate cells in a pellet. Calcite


a)
b)
c)
d)
all species moderate light
G. oceanica
C. leptoporus
C. pelagicus
E. huxleyi
U. sibogae fol.
U. sibogae sib.
O. fragilis
A. robusta
C. pel. hyal. (HOLO)
H. carteri
ε18O(calcite-water)
δ18Ocalcite (PD B) - δ18Owater (SMOW)
δ18Ocalcite (PDB) - δ18Owater (SMOW)
calcite production rate (pg/cell/day)
growth rate
cell diameter (μm)
Surface area/volume ratio

production of the cells in culture was estimated by dissolving a split of the harvested coccolith pellet and measuring the Ca concentration via flame atomic absorption spectroscopy. We estimate the rate of organic C uptake by the cells, from calculated organic C quotas of the cells times the cell division rate. We assume that organic C quota is proportional to biovolume and use relationships from experiments with *Emiliania huxleyi* indicating typical cell quotas of 20 pg organic C/cell with cell diameters of approximately 4 μm [8]. Cell diameters were measured with an eyepiece graticule during the culture experiments and cell volume and surface area were calculated assuming spherical geometry for all species except for *Algirosphaera robusta*, for which elliptoid geometry was assumed.

When the culture was harvested, a filtered medium sample was collected and poisoned with HgCl to measure the stable isotope composition of the medium. No $\delta^{13}C$ dissolved inorganic carbon (DIC) data are available for light or temperature experiments because tubes froze and cracked on the way to the analytical lab and were contaminated with atmospheric CO_2, but reliable $\delta^{13}C$ DIC data were obtained for the multi-species experiments. $\delta^{18}O$ medium data were obtained for both multi-species and light experiments and ranged from −0.3 to 1.42‰ (SMOW).

2.2. Analyzing stable isotopes

Stable isotopes were subsequently measured using a Finnigan 252 mass spectrometer, equipped with an automated carbonate extraction line (Kiel device) at the Free University of Amsterdam. The reproducibility of a routinely analyzed carbonate standard (NBS 19) is better than 0.05‰ for $\delta^{13}C$ and 0.09‰ for $\delta^{18}O$ (1σ). Based on replicate analyses of splits of culture samples, the mean reproducibility of $\delta^{18}O$ and $\delta^{13}C$ in coccolith calcite is 0.1‰ for both.

$\delta^{18}O$ water samples were measured at the Free University of Amsterdam on Finnigan Delta+ mass spectrometers, 1σ of water samples was ±0.15‰ for $\delta^{18}O_w$. $\delta^{13}C$ was measured at the Alfred Wegener Institute Bremerhaven on a Finnigan MAT252 with GASBENCH I (1σ < 0.1‰ $\delta^{13}C_{DIC}$). Reproducibility is based on a routinely run lab water standard. From these data, we calculate the isotopic fractionation between coccolith calcite and water. The oxygen isotopic fractionation between calcite and seawater or culture medium is typically reported as:

$$\varepsilon^{18}O_{(\text{calcite}-H_2O)} = [\alpha_{(\text{calcite}-H_2O)} - 1] \times 1000$$

$$= [(\delta_{\text{calcite}} + 1000)/(\delta_{H_2O} + 1000)] \times 1000$$

For carbon isotopes, we report $\varepsilon^{13}C$ between calcite and medium DIC. For fractionation factors (α) close to 1, as is the case between calcite and medium DIC, $\varepsilon_{(\text{calcite}-H_2O)}$ differs only slightly from $\delta_{\text{calcite}} - \delta_{DIC}$. Some workers discuss $\delta^{18}O_{\text{calcite}} - \delta^{18}O_{H_2O}$, and in some cases, workers have reported $\delta^{18}O_{\text{calcite (PDB)}} - \delta^{18}O_{H_2O\text{ (SMOW)}}$. This latter expression is simple to calculate but it is not ideal since it mixes units and obscures the large fractionation between calcite and water. Consequently, we have chosen to express the observed fractionation as ε for both carbon and oxygen isotopes, but also show the $\delta^{18}O_{\text{calcite (PDB)}} - \delta^{18}O_{H_2O\text{ (SMOW)}}$ on figures to facilitate comparison

←

Fig. 1. Oxygen isotopic fractionation in coccolith calcite from eight coccolithophorid species (and two variations of *U. sibogae* and two phases of the life cycle of *C. pelagicus*) at constant temperature of 17°C and moderate light conditions. Fractionation is expressed as ε and as the isotopic difference between water (SMOW) and calcite (PDB), to facilitate comparison with results of Dudley et al. [1–4]. The latter expression is 0.27‰ higher than the alternative convention expressing the isotopic difference between calcite and water both on the PDB scale [23]. (a) Oxygen isotope fractionation vs. calcification rate. (b) Oxygen isotopic fractionation vs. cell division rate (day^{-1}). The solid line is the least squares fit: $y = 3.44x + 28.75$, $r^2 = 0.84$. (c) Oxygen isotopic fractionation vs. cell diameter. Oxygen isotopic fractionation for cultures of [3], shown in black symbols, was calculated by solving their paleotemperature equation for each species at 17°C, and using typical cell sizes for the species they cultured. Polynomial fit ($y = 0.05x^2 - 1.38x + 38.93$, $r^2 = 0.78$) excludes data from *C. pelagicus*. (d) Oxygen isotopic fractionation vs. cell surface area/volume ratio. In panels c and d, additional results and symbols for cultures from [3] are for: *Syracosphaera pulchra* (x), *Cricosphaera* (*Pleurochrysis*) *carterae* (*), *Umbilicosphaera hurlburtiana* (+), and *Reticulofenestra sessilis* (–). Box shows estimated equilibrium $\varepsilon^{18}O$ between carbonate and seawater at 17°C is 30.6 according to the equation [9].

with previous culture data. This fractionation is the effective fractionation factor between seawater and coccolith calcite, but may be the net result of several steps of isotopic fractionation from seawater to the site of coccolith calcification.

3. Results

3.1. Interspecific differences in stable isotope composition

Among the different species cultured, both oxygen and carbon isotopes show a large range of 'vital effects' in coccolith calcite. The magnitude of these effects is similar to those observed in previous culture experiments [3].

For oxygen isotopes, we observe a 5‰ range in $\varepsilon^{18}O$ ($\approx \delta^{18}O_{calcite} - \delta^{18}O_{medium}$), with values both above and below the expected equilibrium $\varepsilon^{18}O$ for precipitation from seawater at 17°C. $\varepsilon^{18}O$ is not correlated to different rates of calcite precipitation in different species (Fig. 1a), nor to different rates of organic carbon fixation in different species (Table 2). $\varepsilon^{18}O$ is highly correlated with different maximum growth rates of the different species under moderate light and nutrient-replete conditions (Fig. 1b), and is moderately correlated with the size of the cell and the cell's surface area/volume ratio (Fig. 1c, d). The one exception to the latter pattern is *C. pelagicus*, which shows a higher growth rate than expected for its cell size compared to the other cultured species. This general pattern, of species with larger cells showing lower $\varepsilon^{18}O$, is consistent with culture results of [3] whose smaller cells (including *E. huxleyi*, *G. oceanica*, and *Reticulafenestra sessilis*) had higher $\varepsilon^{18}O$ than larger cells (*C. leptoporus*, *U. sibogae*, *Umbilicosphaera hurlburtiana*, *Syracosphaera pulchra*, and *Cricosphaera carterae*; Fig. 1c).

The interspecific differences in stable isotope fractionation do not appear to be correlated with major phylogenetic divisions of coccolithophorids, although the species with the highest $\varepsilon^{18}O$, *G. oceanica* and *E. huxleyi*, are closely related. Culture results from a small-celled, fast-growing species which is not related to *E. huxleyi* or *G. oceanica* would be important to establish whether this relationship reflects their common phylogeny or merely their small cell size and fast growth rate.

Differences in oxygen isotopic fractionation among different species do not correlate with major biogeographical differences. *C. leptoporus*, *G. oceanica*, and *E. huxleyi* show a range of fractionation yet all are cosmopolitan, placolith-bearing and have similar biogeography. Conversely, similar isotopic fractionation is observed in *H. carteri*, typically a warm-water dweller, and *C. pelagicus*, which typically prefers colder waters. Heterococcoliths and holococcoliths, which characterize the diploid and haploid life phases of *C. pelagicus*, respectively, show distinct $\varepsilon^{18}O$ values which both fall on the trend of $\varepsilon^{18}O$ vs. growth rate (Fig. 1b).

For carbon isotopes, we observe a 5‰ range in $\varepsilon^{13}C$ ($\approx \delta^{13}C_{calcite} - \delta^{13}C_{medium\ DIC}$), with values both above and below the expected equilibrium $\varepsilon^{13}C$ for precipitation from seawater at 17°C. As

Table 2
Matrix of correlation coefficients (ρ) between $\varepsilon^{18}O$ and species-specific growth rate (day^{-1}), calcification rate (pg calcite $cell^{-1}$ day^{-1}) and carbon fixation rate (pg org C $cell^{-1}$ day^{-1}), cell size, and cell surface area/volume ratios

	$\delta^{18}O$	$\delta^{13}C$	Growth rate	Calcification rate	C_{org} fixation rate	Cell diameter	Surface area/volume
$\delta^{18}O$	–	–	–	–	–	–	–
$\delta^{13}C$	0.86	–	–	–	–	–	–
Growth rate	0.92	0.91	–	–	–	–	–
Calcification rate	−0.21	−0.34	−0.06	–	–	–	–
C_{org} fixation rate	−0.07	−0.37	0.66	0.83	–	–	–
Cell diameter	−0.59	−0.68	−0.46	0.81	0.82	–	–
Surface area/volume	0.77	0.91	0.66	−0.70	−0.66	−0.94	–

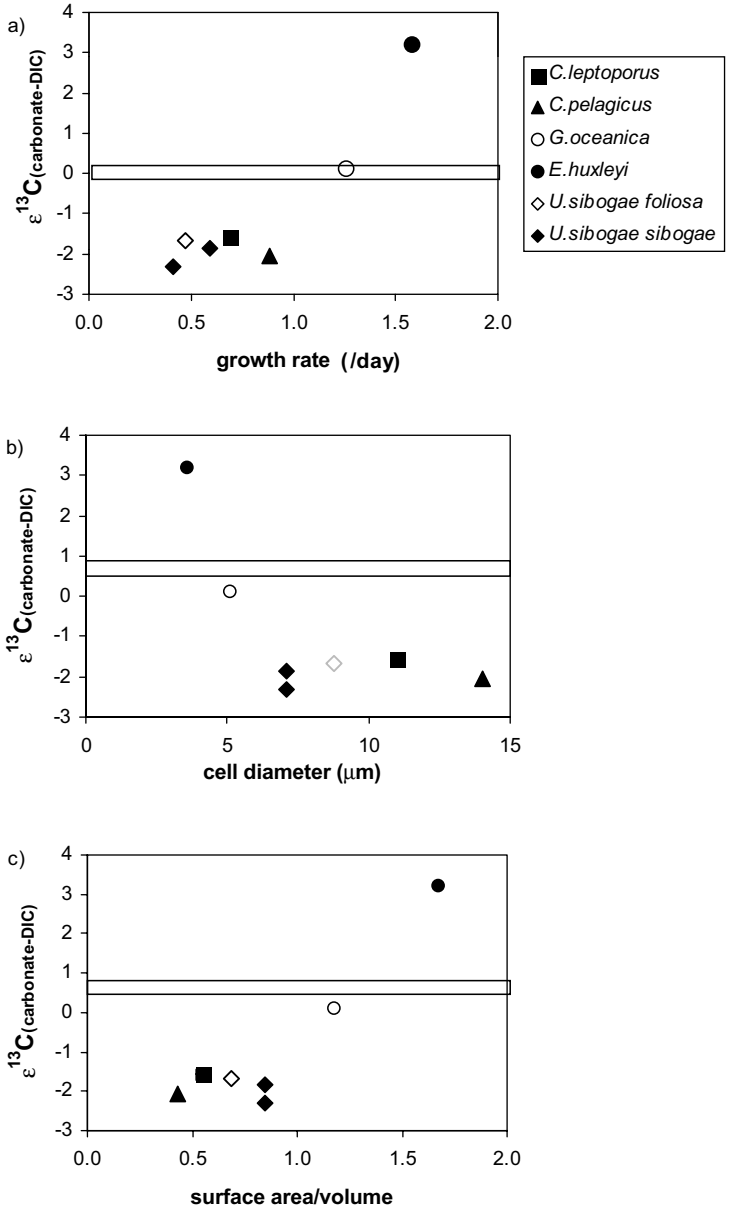


Fig. 2. Carbon isotopic fractionation in coccolith calcite from eight coccolithophorid species (and two variations of *U. sibogae* and two phases of the life cycle of *C. pelagicus*) at constant temperature of 17°C and light saturating conditions. (a) Carbon isotopic fractionation vs. cell division rate (day^{-1}). (b) Carbon isotopic composition vs. cell diameter. (c) Carbon isotopic fractionation vs. cell surface area/volume ratio. The isotopic composition of the coccoliths is reported as $\varepsilon^{13}C$ ($\approx \delta^{13}C_{calcite} - \delta^{13}C_{medium\ DIC}$). Box shows estimated equilibrium $\varepsilon^{13}C$ between dissolved bicarbonate and calcite at 17°C.

was the case for oxygen isotopes, $\varepsilon^{13}C$ is not significantly correlated with different rates of organic carbon fixation in different species nor with different rates of calcite precipitation in different species (Table 2). $\varepsilon^{13}C$ is strongly correlated with both the different maximum growth rates of the different species and the cell size and surface area/volume ratio (Fig. 2). However, at growth rates

slower than 1 cell day^{-1} (or cells larger than 7 μm) there is no further depletion in $\varepsilon^{13}C$.

The vital effects in carbon and oxygen isotopes are moderately correlated but also show a break point where oxygen isotope ratios continue to vary but carbon isotope ratios are relatively constant (Fig. 3).

3.2. Intraspecific isotopic variations with growth rate

In experiments with single species, there is a much more limited range of $\varepsilon^{18}O$ values. For *G. oceanica*, decreasing irradiance reduced growth rates from 1.9 to 0.5 cells day^{-1}, yet $\varepsilon^{18}O$ varied by less than 1‰ (Fig. 4). Likewise, for *C. leptoporus*, decreasing irradiance reduced growth rates from 0.7 to 0.2 cells day^{-1}, yet $\varepsilon^{18}O$ varied only 1.5‰ (Fig. 4).

3.3. Temperature dependence of oxygen isotopic fractionation

$\varepsilon^{18}O$ in *G. oceanica* and *H. carteri* show a strong temperature dependence (Fig. 5). Temperature-induced variations in growth rate also do not affect $\varepsilon^{18}O$ in cultures of *G. oceanica* or *H. carteri*. In *G. oceanica*, with increasing temperature, growth rate increased from 1.1 to 2.0 cells day^{-1}, yet the slope of the relationship of $\varepsilon^{18}O$ with temperature is indistinguishable from that measured for equilibrium precipitation [9]. If increasing growth rate caused $\varepsilon^{18}O$ in *G. oceanica* to increase at the same rate it does among different species, the growth rate effect would compensate for the $\varepsilon^{18}O$ decrease with temperature for all except the hottest experiment and there would be no correlation between $\varepsilon^{18}O$ and temperature (Fig. 5). Despite different growth rates for a given temperature, the multiple strains of *G. oceanica* isolated from widely different oceanographic settings yield a remarkably consistent paleotemperature relationship. The difference between the *G. oceanica* paleotemperature equation calculated from our cultures and that of [3] is within analytical uncertainties.

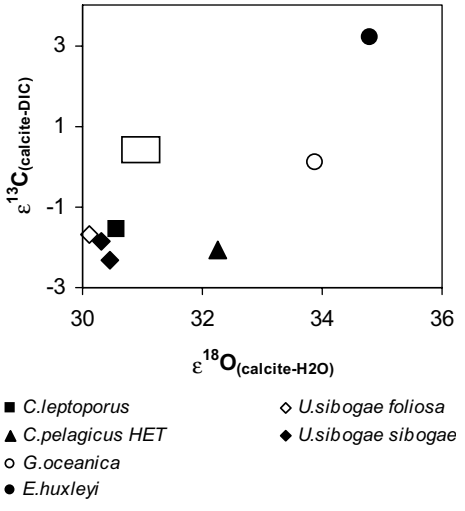


Fig. 3. Comparison of coccolith $\varepsilon^{18}O$ and $\varepsilon^{13}C$ data from our experiments; box shows estimated equilibrium compositions as given in Figs. 1 and 2.

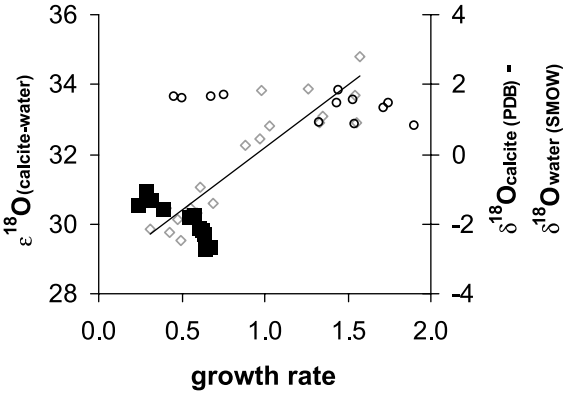


Fig. 4. $\varepsilon^{18}O$ of *G. oceanica* and *C. leptoporus* under variable irradiance and growth rate (symbols as in Fig. 1) compared with the growth rate effect among different species at constant irradiance as shown in Fig. 1b (open diamonds with best fit line from Fig. 1a). All experiments at constant temperature of 17°C. For a single species, growth rate and calcification rate are highly correlated ($r \approx 0.9$) so trends of $\varepsilon^{18}O$ vs. calcification rate for *G. oceanica* and *C. leptoporus* in light experiments are nearly identical to those shown here.

4. Origin of interspecific vital effects

Interspecific variations in $\varepsilon^{18}O$ are most highly correlated with growth rate. However, light-modulated or temperature-modulated growth rate variations do not induce comparable $\varepsilon^{18}O$

variations in a single species, suggesting that growth rate is not the ultimate control over vital effects in coccoliths. The data from multiple growth rates for a single species also indicate that there is nothing unique about the slope of the growth rate vs. $\varepsilon^{18}O$ relationship in species grown at 17°C (Fig. 1b). There is a greater range of growth rates among the small, fastest-growing species, so with reduced irradiance the same variation in $\varepsilon^{18}O$ would occur over a smaller range of growth rates, causing a steeper slope. Instead these vital effects must reflect some other physiological adaptation correlated with growth rate differences among species. The vital effects were moderately to strongly correlated with cell size or surface area/volume ratio (Fig. 1), which varies among species but which would not change significantly (i.e. by more than 20%) in a single species across a broad range of culture conditions. The correlation between cell size and growth rate may reflect cell growth limited by the cell's rate of acquisition of nutrients or C across cell membranes (e.g. [10]) where larger cells with lowest surface area/volume ratios may grow more slowly.

The fact that there are consistent relationships between the vital effects and cell size and growth rate across different species suggests that vital effects may arise from a consistent type of mechanism in all species. If coccolith vital effects resulted from a random array of different processes in different organisms it is unlikely that there would be a systematic, first order relationship with cell size and growth rate. For coccolithophorids, which calcify intracellularly in specialized vesicles, the challenge lies in ascertaining how kinetic and thermodynamic processes of isotopic fractionation are linked to cellular carbon acquisition and carbonate precipitation. This is a daunting challenge since studies have not conclusively distinguished whether C is taken up only as CO_2 by passive diffusion or also by active transport of CO_2 or HCO_3^- (e.g. [11,12]). In reality, the patterns of stable isotopic variations in coccoliths

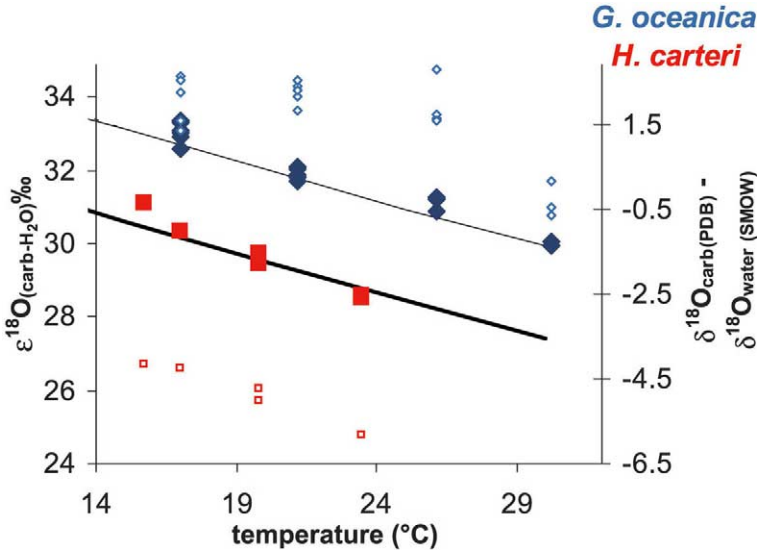


Fig. 5. $\varepsilon^{18}O$ vs. temperature for *G. oceanica* (black diamonds) and *H. carteri* (gray squares) showing equilibrium paleothermometry relation of [9] (solid line) which has the same slope as that of the *G. oceanica* (dashed line) and *H. carteri* data. Miniature diamonds and squares (for *G. oceanica* and *H. carteri*, respectively) indicate the expected $\varepsilon^{18}O$ if in addition to the temperature effect, there was also a growth rate effect on $\varepsilon^{18}O$ comparable in magnitude to that observed among different species as in Fig. 1. Equilibrium paleothermometry relation of [9] is given by: $1000\times\ln\alpha=(18.03\times10^3\times T^{-1})-32.42$, where temperature ($T$) is in degrees Kelvin. Values are also plotted on the scale of $\delta^{18}O_{calcite\ (PDB)}-\delta^{18}O_{water\ (SMOW)}$, as used by [1,3]. Equations for the least squares fit in units of $\delta^{18}O_{calcite\ (PDB)}-\delta^{18}O_{water\ (SMOW)}$, are $y=4.93-0.22x$ for *G. oceanica* and $y=3.53-0.28x$ for *H. carteri*.

may provide more constraints for unraveling the cellular C and acquisition methods than vice versa.

Three potential effects: (1) kinetic fractionation during calcite precipitation or cellular uptake, (2) fractionation of the intracellular C pool during photosynthesis, and (3) pH-dependent fractionation, may contribute to the isotopic variations observed in coccoliths.

1. Kinetic effects on isotopic fractionation during carbonate precipitation are the most widely invoked mechanism for vital effects in biogenic carbonates [13,14]. If the precipitation of carbonate is faster than the hydroxylation of carbon dioxide ($CO_2+OH^- \rightarrow HCO_3^-$), then the faster hydroxylation of ^{12}C- and ^{16}O-bearing CO_2 produces covarying depletions in both $\delta^{18}O$ and $\delta^{13}C$ of the carbonate compared to equilibrium. The most rapidly precipitated carbonate is isotopically lighter for both oxygen and carbon. Kinetic effects are not likely to be important where carbonic anhydrase catalyzes equilibrium between carbonate species and water. While carbonic anhydrase has been identified in the chloroplasts of some coccolithophorid species, its location relative to the uptake and calcifying reactions may be important. If it is localized at the site of one reaction but not another, kinetic effects may still play a role in coccolith carbonate. However, kinetic vital effects cannot produce carbonates which are isotopically enriched in ^{13}C or ^{18}O with respect to equilibrium calcite, as are observed for coccoliths from some species in these cultures and those of [3].
2. The carbon isotopic composition of coccoliths might be influenced by the strong kinetic isotopic fractionation during photosynthesis if there were significant exchange between the carbon pools used for photosynthesis and calcification [12]. The residual carbon dioxide not fixed through photosynthesis would be isotopically heavier in carbon, since ^{12}C is preferentially fixed in organic carbon. Conversely, respired CO_2 would be isotopically lighter than CO_2 diffused across the cell membrane. Addition of residual C from photosynthesis could explain the $\delta^{13}C$ values higher than equilibrium calcite but could not explain enriched $\delta^{18}O$ values.
3. The different equilibrium fractionations among different carbonate species with respect to water may also give rise to important variations in the isotopic compositions of carbonates (e.g. [5,15]. In oxygen isotopic fractionation between CO_2 and carbonate species and water, CO_3^{2-} is enriched by 18‰, HCO_3^- is enriched by 34‰, and CO_2 is enriched by 42‰. Because of the large (16‰) difference in the oxygen isotopic composition of carbonate and bicarbonate, whose relative abundance depends on the pH, $\varepsilon^{18}O$ can be set by the pH if the precipitating carbonate uses HCO_3^- and CO_3^{2-} in their relative abundance in the calcifying fluid precipitates all dissolved inorganic carbon. However, to explain the 5‰ range of $\varepsilon^{18}O$ observed among different coccolith species exclusively through this mechanism would require a pH variation of nearly 4 pH units, unrealistically large. A small pH effect on $\varepsilon^{18}O$ might be superimposed on other sources of isotopic variation, perhaps explaining the higher than equilibrium $\varepsilon^{18}O$ by lower pH in the smaller species. If coccolithophorids actively take up HCO_3^-, a lower pH might reflect a smaller alkalinity or HCO_3^- flux relative to the larger diffusive CO_2 flux permitted by the high surface area/volume ratio of the small species. The carbon isotopic composition of HCO_3^- is about 0.5‰ heavier than that of CO_3^{2-} and both are significantly heavier than CO_2 (by about 9‰). Consequently, if coccolithophorids actively take up HCO_3^-, it may affect the carbon isotopic composition of coccoliths as well. Cell size, and its constraints on the relative importance of passive CO_2 diffusion vs. active HCO_3^- uptake, may be important controls on coccolith stable isotope vital effects.

Integration of these three potential sources of stable isotopic variations in coccolith calcites will require numerical models of stable isotopic fractionation with cell growth similar to those which have been developed to simulate carbon isotope fractionation in algal organic matter (e.g. [11]) or isotope effects in foraminifera (e.g. [16]). Such

models may be able to account for the likely dependence of kinetic fractionation on the surface area/volume ratio which sets the diffusive CO_2 influx/outflux and hence the proportion of available C fixed by the cell, as well as other effects.

5. Implications for reconstructing temperature and seawater chemistry from coccolith stable isotopes

The constancy (to within 1‰) of species-specific oxygen isotopic vital effects among multiple strains of the same species isolated from a wide range of oceanographic settings and even over large changes in cell division rates and calcification rates confirms that in downcore records, vital effects of a given species can be assumed to vary by much less than the interspecific vital effect range. Smaller variations in vital effects may still arise from changes in cell biochemistry with changes in seawater pH or nutrients and should be investigated in future studies.

Our culture results may provide the first species-specific correction factors for carbon isotopic vital effects in coccoliths, although further experiments are needed to confirm that these effects are constant for each species. To date, limited data exist only for *E. huxleyi*. In light-regulated cultures of *E. huxleyi* spanning an array of growth rates, the carbon isotopic fractionation of coccoliths showed only limited variation (less than 2‰; most samples within a 1‰ range) and no correlation with growth or calcification rate [17]. Much more variable carbon isotopic fractionation was inferred for *E. huxleyi* coccoliths in another set of light-regulated growth experiments [18]. However, this variability may in part reflect analytical uncertainties, since in the latter experiment coccolith isotopic composition was not measured directly, but estimated from measurements of isotopic fractionation in total particulate carbon and particulate organic carbon [18].

Confirmation of a large range of interspecific vital effects in both carbon and oxygen isotopes implies that the effects of changing nannofossil assemblages are significant. For Pleistocene coccolith-dominated bulk or polyspecific coccolith records, the relative carbonate contribution of each species could be balanced by a species-specific correction factor, as demonstrated by [4], as long as the contribution of non-coccolith material is also constrained.

The inability to determine species-specific vital effects in extinct species has been the largest challenge to paleoceanographic application of coccolith stable isotopes in older sediments. For example, [19] suggested that the $\delta^{13}C$ shift in coccolith-dominated bulk carbonate at the onset of the Paleocene/Eocene event could be largely a response to a shift in nannoplankton assemblages rather than a shift in surface water $\delta^{13}C$ as interpreted by [20]. The relationship between coccolith isotopic vital effects and cell size (and cell surface area/volume) may provide a crude basis for estimating the vital effects in extinct species. For many time intervals, whole coccospheres (cell plus its surrounding covering of coccoliths) are preserved, although rare. It would be possible to estimate the cell diameter from measurements on coccospheres. At present, this approach would give only approximate (± 1‰ for $\varepsilon^{18}O$ and ± 0.5‰ for $\varepsilon^{13}C$) estimates of vital effects due to scatter in the cell size/growth rate relationship. However, over times of large species turnover and large isotopic shifts (K/T boundary, Paleocene/Eocene event and other methane hydrate liberation events) it may provide a way to constrain the extent to which changes in bulk or fine fraction $\delta^{13}C$ or $\delta^{18}O$ could be attributed to nannoplankton assemblage changes.

New decanting, density stratified columns and microfiltering techniques [21,22] have permitted separation of more restricted (in cases nearly monospecific) coccolith fractions from sediments. Although the methods are time-intensive, they remove the necessity for correcting isotopic records for changing nannofossil assemblages and may be the best approach for older sediments where species-specific vital effects cannot be determined in culture. The potential to separate monospecific coccolith fractions from sediments also offers, for the first time, the possibility of using the organic and inorganic C phase of the same organism (*G. oceanica*) to calibrate temperature proxies, based on alkenone undersaturation ratios and

species-specific $\delta^{18}O$ of coccolith calcite. Also, if the constancy of carbon isotopic fractionation with growth rate can be confirmed, then estimates of past carbon isotopic fractionation during photosynthesis (ε_p) might be more reliably obtained from the isotopic difference between alkenone biomarkers and the coccoliths of the alkenone producers (*E. huxleyi* and *G. oceanica*) rather than from fractionation between biomarkers and the carbonate of foraminifera as is current practice. Finally, if models show that coccolith stable isotope fractionation constrains mechanisms of carbon acquisition and calcification in coccolithophorids, measurements of coccolith stable isotopes in the past might elucidate how these mechanisms have changed in the past.

6. Conclusions

We have confirmed a large (5‰) range of oxygen isotopic fractionation among different species of coccolithophorids and demonstrated that a comparably large range in isotopic fractionation exists for carbon isotopes in coccoliths. These interspecific vital effects correlate with cell size and growth rate. However, within a single species, even among different strains, vital effects are constant to within 1‰, with no strong response to growth rate variations.

The constancy of vital effects suggests that coccolith stable isotopes will provide reliable phase for paleoceanographic reconstruction of temperature and seawater chemistry, as long as monospecific fractions are used or changes in nannofossil assemblages are accounted for with species-specific correction factors. These culture results provide the first species-specific correction factors for carbon isotopes in coccoliths.

These culture data provide new constraints on the origins of vital effects in coccoliths. We suspect that the cell size, and its constraints on the rate of CO_2 diffusion relative to carbon fixation, may be a first order influence on these vital effects. However, more rigorous modeling of isotopic effects for different carbon and alkalinity uptakes, including both equilibrium and potential kinetic effects, is needed to arrive at a quantitative understanding of mechanisms of vital effects in coccolith carbonate. With the aid of quantitative models, such as those developed for isotopic fractionation in algal organic matter [11], stable isotopic fractionation in coccoliths may provide important constraints on mechanisms of carbon acquisition of coccolithophorids in both modern and extinct species.

Acknowledgements

This project was supported in part by the EC-TMR project CODENT – Coccolithophorid Evolutionary Biodiversity and Ecology Network (FRMX-ET97-0113) and in part by Williams College. We thank Jesse Adkins, Luc Beaufort and an anonymous reviewer for the constructive comments on the manuscript, A. Mackenson and H. Vonhof for instrument time for isotopic analyses, and Klaus Keller and Ted McConnaughey for valuable discussions about mechanisms of isotopic fractionation. We thank D. Kroon, P.G. Mortyn and F. Peeters for review of earlier versions of the manuscript. This is a Netherlands School for Sedimentary Geology (NSG) publication number 20030306.***[BARD]***

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