ABSTRACT
The magnesium/calcium ratio (Mg/Ca) and calcium (Ca) concentration of seawater have oscillated throughout geologic time; our experiments indicate that these variables have strongly influenced biomineralization and chalk production by coccolithophores. The high Mg/Ca ratio of modern seawater favors precipitation of high-Mg calcite and/or aragonite. In contrast, the low Mg/Ca ratio of imputed Cretaceous seawater favored precipitation of low-Mg calcite. We have discovered that some coccolithophore species today secrete skeletal elements of high-Mg calcite, rather than low-Mg calcite, as conventionally believed. These species incorporated less Mg when the ambient Mg/Ca ratio was lowered, secreting low-Mg calcite in imputed Cretaceous seawater. Calcification stimulates coccolithophore population growth by contributing CO$_2$ to photosynthesis. Three extant coccolithophore species multiplied much faster as the composition of ambient seawater was shifted toward that estimated for Cretaceous seas. Two of these species secreted high-Mg calcite in ambient seawater having Mg/Ca > 1, and incorporation of Mg in a calcite crystal inhibits growth. Calcification of the third species, which secreted low-Mg calcite at all ambient Mg/Ca ratios, is hindered by the high Mg/Ca ratio and low absolute concentration of Ca of modern seawater. We conclude that the ionic composition of Cretaceous seawater enabled coccolithophores to produce massive chalk deposits, and conversely, that the ionic composition of modern seawater inhibits population growth for most extant coccolithophore species, which occupy nutrient-poor waters and fail to respond to fertilization by nitrate, phosphate, or iron.

Keywords: coccolithophore, limestone, chalk, calcite, high-Mg calcite.

INTRODUCTION
Coccolithophores (calcareous nannoplankton) are unicellular, spheroidal planktonic algae that play a significant role in food webs of the modern ocean. These algae secrete coccoliths, which are shield-like elements of calcite, typically 1–10 μm in diameter, that are produced in the Golgi body and extruded to cover the cell surface. Coccolithophores are classified as nannoplankton because of their small size (mostly <20 μm). They were much more productive during Late Cretaceous time than today, producing the voluminous chalk deposits that gave the Cretaceous Period its name. We have conducted experiments that support the hypothesis (Stanley and Hardie, 1998, 1999) that the composition of Cretaceous seawater permitted coccolithophores to flourish, whereas the composition of modern seawater retards their productivity.

There have been three intervals during the Phanerozoic Eon when shallow-marine nonskeletal carbonate precipitates have consisted of aragonite and high-Mg calcite, and two intervals when they have consisted of low-Mg calcite (Sandberg, 1985). Oscillations in the Mg/Ca ratio of seawater have produced these mineralogical shifts (Hardie, 1996). Laboratory experiments indicate that low-Mg calcite (<4 mol% Mg substituting for Ca) precipitates from seawater when the Mg/Ca mole ratio is <1, high-Mg calcite precipitates when this ratio is >1, and aragonite precipitates along with high-Mg calcite when the ratio is >2 (Füchtbauer and Hardie, 1976, 1980). Furthermore, marine hydrothermal brine/river flux mass-balance calculations (Hardie, 1996), fluid inclusions (Lowenstein et al., 2001), and bromine concentrations in halite (Siemann, 2003) indicate that transitions between aragonite and calcite seas have coincided with shifts in the Mg/Ca ratio of seawater to above or below 2, as predicted from experiments. Calcifying marine organisms exemplify the same pattern. Most biologically simple taxa that have functioned as major carbonate reef builders or producers of carbonate sediment have secreted skeletons consisting of minerals favored by ambient seawater chemistry (Stanley and Hardie, 1998, 1999).

COCCOLITHOPHORE CALCIFICATION AND PRODUCTIVITY
The molar Mg/Ca ratio (≤1) and absolute concentration of Ca (~25–30 mM) in Late Cretaceous seawater were close to their minimum and maximum values, respectively, for the Phanerozoic Eon. We have hypothesized that these conditions accounted for the unique formation of massive, widespread Upper Cretaceous chalk deposits through the accumulation of calcitic coccoliths (Stanley and Hardie, 1998, 1999). Such chalks are present in North America, Europe, the Middle East, and Australia (Hattin, 1988). Despite existing at high taxonomic diversity, coccolithophores failed to form comparable chalk deposits in the Early Cretaceous or post-Cretaceous time. Nearly 300 coccolithophore species exist today (Winter and Seisser, 1994). Late Cretaceous chalk accumulated at estimated depths of 100–500 m in warm epicontinental seas (Scholle, 1977). Such seas persisted in Europe and the Middle East throughout Paleogene time, but accumulated moderate volumes of chalk only during the Paleocene, when Mg and Ca concentrations in seawater remained close to Cretaceous levels.

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phores’ calcification and therefore inhibits population growth (Riebesell et al., 2000).

Given the importance of CO₂ fertilization from calcification, we hypothesized that the high Mg/Ca ratio and low calcium concentration of modern seawater limit population growth rates of most coccolithophore species in the modern ocean—species that may not pump calcium as effectively as the newly evolved Emiliania and Gephyrocapsa.

EXPERIMENTS ON THE EFFECTS OF SEAWATER CHEMISTRY

We conducted experiments to test the effects of the Mg/Ca ratio and absolute concentration of Ca in seawater on the population growth rate of three coccolithophore species that represent a variety of ecological adaptations: Pleurochrysis carterae, a nearshore species that blooms in the presence of abundant nutrients, although less prolifically than E. huxleyi (Brand, 1991); Coccolithus neohelis, a nearshore species that does not bloom; and Ochrosphaera neopolitana, an oceanic species that is confined to low-nutrient oceanic environments where it cannot bloom.

Our experiments employed Mg/Ca ratios and absolute concentrations of Ca for Cretaceous and post-Cretaceous intervals produced by marine hydrothermal brine/river flux mass-balance calculations (Hardie, 1996; Stanley and Hardie, 1998).

Changes in Mineralogy with Changes in Seawater Chemistry

Although the conventional view is that coccolithophores secrete low-Mg calcite in modern seawater (Siesser, 1977), Coccolithus neohelis was the only one of our experimental species to secrete low-Mg calcite in all seawater treatments (Fig. 1A). In contrast, Pleurochrysis carterae and Ochrosphaera neopolitana secreted high-Mg calcite in seawater of modern composition. Like coralline algae (Stanley et al., 2002), echinoids, crabs, shrimp, and serpulid worms (Ries, 2004), these species incorporated progressively less Mg in their skeletons as the ambient Mg/Ca ratio was reduced from that of modern seawater. They departed only slightly from the fractionation pattern for nonskeletal calcite, secreting low-Mg calcite in imputed Cretaceous seawater (Figs. 1B, 1C).

Population Growth Rates and Chalk Production

Coccolithus neohelis would be expected to calcify and multiply progressively more rapidly as the ambient Mg/Ca ratio is lowered because it secretes low-Mg calcite at all Mg/Ca ratios and this mineral is favored by low ratios. Although Pleurochrysis carterae and Ochrosphaera neopolitana secrete calcite with Mg percentages that are more compatible with ambient seawater chemistry, they too would be expected to secrete coccoliths more effectively and multiply more rapidly with a decrease in the ambient concentration of Mg, because incorporation of Mg reduces the rate of step growth in a calcite crystal (Davis et al., 2002). In addition, reduction of the absolute concentration of Ca when seawater is shifted from Cretaceous to modern composition would be expected to retard population growth for all coccolithophore species, because Ca is the dominant cation even in high-Mg calcite. In other words, regardless of whether a given coccolithophore species incorporated more Mg in its calcite as the Mg/Ca ratio of seawater rose after Cretaceous time and the absolute concentration of Ca declined, it should have calcified and multiplied more slowly.

As predicted, stepwise reduction of the ambient Mg/Ca ratio and concomitant increase in the absolute concentration of Ca from modern seawater values to Cretaceous values resulted in a marked increase in population growth rates for all three of our experimental species (Figs. 2 and 3). These results are robust in that the higher growth rates were

![Figure 1. Magnesium in calcite (mol%) secreted by three species of coccolithophores as function of molar Mg/Ca ratio of artificial seawater in which they were grown. Error bars are standard deviations of five measurements. Fitted curves are power functions. Dashed lines depict pattern for inorganic precipitation (Füchtbauer and Hardie, 1976, 1980).](image1)

![Figure 2. Exponential population growth and chalk production for Pleurochrysis carterae as function of ambient Mg/Ca molar ratio and Ca concentration of seawater. For modern seawater, Mg/Ca molar ratio is 5.2 and absolute concentration of Ca is 0.2 mM; for imputed Late Cretaceous seawater, Mg/Ca molar ratio is between 0.5 and 1, and absolute concentration of Ca is between 42.2 and 25.3 mM. Inset displays total calcite production for each culture of P. carterae after 7 days. Spearman rank correlation (r_s) = 0.99 for both growth rates and calcite production.](image2)
the imputed Cretaceous Mg/Ca ratio, its exponential rate of population growth approximately quadrupled (Fig. 3C).

For all three sets of data, Spearman rank correlation ($r_s$) = 0.99. D: Increase in size and robustness of coccoliths of Ochrosphaera neopolitana when grown in seawater of Cretaceous composition (scale bar = 1 μm).

Figure 3. Effects of ambient Mg/Ca ratio on growth of three coccolithophore species. A–C: Exponential rates of population growth ($r$) for species as function of Mg/Ca molar ratio and absolute concentration of Ca in ambient seawater. Fitted curves in B and C are exponential, whereas that in A is linear. For all three sets of data, Spearman rank correlation ($r_s$) = 0.99. D: Increase in size and robustness of coccoliths of Ochrosphaera neopolitana when grown in seawater of Cretaceous composition (scale bar = 1 μm).

Figure 4. Dependence of population growth rate of Pleurochrysis carterae on both Mg/Ca ratio and absolute concentration of Ca in ambient seawater. A: With Mg/Ca molar ratio held constant at 3.5, elevation of Ca concentration from 14.1 mM to 25.3 mM produced faster population growth. B: With Mg/Ca molar ratio held constant at 1.0 (Late Cretaceous level), reduction of absolute concentration of Ca from Late Cretaceous level of 31.6 mM to modern seawater level of 10.2 mM produced slower population growth. C: With Ca held constant at 10.2 mM (concentration in modern seawater), reduction of Mg/Ca molar ratio from 5.2 (ratio for modern seawater) to 1.0 (Late Cretaceous ratio) produced faster population growth. D: With absolute concentration of Ca held constant at 25.3 mM (Early Cretaceous level), elevation of Mg/Ca molar level from Early Cretaceous level of 1.5 to aragonite sea level of 3.5 produced slower population growth. Experiments with Coccolithus neohelis yielded similar results.

LIMITING FACTORS FOR POPULATION GROWTH IN THE MODERN OCEAN

Our results imply that the high Mg/Ca ratio and low absolute concentration of Ca in modern seawater are limiting factors for the majority of extant coccolithophore species, i.e., those that occupy warm, nutrient-poor waters. Ochrosphaera neopolitana is one of these oligotrophic species; therefore, we grew it exclusively at low nutrient levels (see Appendix) and found that, when transferred to seawater with the imputed Cretaceous Mg/Ca ratio, its exponential rate of population growth approximately quadrupled (Fig. 3C).

Unlike concentrations of iron, silica, nitrate, and phosphate, which serve as limiting factors for other groups of phytoplankton (Brand, 1991, 1994; Martin et al., 1994), those of Ca and Mg vary little from place to place in the ocean. We conclude that the unfavorable concentrations of Ca and Mg in modern seawater prevent all but a few coccolithophore species from blooming when conventional nutrients are in abundant supply. Most coccolithophore species are restricted to nutrient-deficient subtropical and/or tropical gyres of the ocean, where they exist at relatively low abundance in what amounts to a refugium.

APPENDIX. METHODS

Coccolithophore strains (CCMP645, CCMP2119, CCMP298) were obtained from the Provasoli-Guillard National Center for Culture...
of Marine Phytoplankton (P-G CCMP). Artificial seawaters were prepared according to the formula of Bidwell and Spotte (1985), but with the Mg/Ca molar ratio varying from 5.2 to 0.5; the molar sum of Mg and Ca was held constant at the level of modern seawater. Cultures were grown in 500 mL of seawater in 1000 mL beakers that were partially covered to reduce evaporation but permit some air circulation. Each beaker was illuminated by a single 120 V, 25 W aquarium lamp at an elevation of 15 cm above the water surface. The beakers sat in a large bath of fresh water maintained at 25 °C by electrical heaters and kept in circulation by aeration. In cultures of *Pleurochrysis carterae* and *Coccolithus neobhei*, concentrations of nitrogen (as nitrate), phosphorus (as phosphate), and iron (as Fe(III)) were, respectively: $8.8 \times 10^{-4}$, $2.3 \times 10^{-4}$, and $1.4 \times 10^{-4}$ mol/L (P-G CCMP culture medium f/2). In cultures of *Ochrosphaera neopolitana*, the corresponding concentrations were lower: $3.5 \times 10^{-5}$, $9.2 \times 10^{-6}$, and $5.8 \times 10^{-7}$, as specified by P-G CCMP (culture medium f/0) for growth of this group of oceanic species. The pH of all cultures remained at 8.15 (±0.1) without any secular trend.

Experiments were carried out over 6–7 d during the initial exponential phase of population growth. Cell concentrations in cultures were measured daily by removing 15 mL of water after homogenizing the upper part of the culture by gentle stirring with a sterile glass rod. Cell density was measured with a Hach 2100P turbidimeter, after which the sample was returned to the culture. The turbidimeter was calibrated by relating measured values linearly to cell concentrations measured with a hemocytometer. Scanning electron microscopic images of culture residues showed that nearly all coccospheres remained intact throughout experiments. Because coccoliths were small relative to cell size and infrequently lost, we are confident that cells constituted nearly all of the suspended material. Percentage of Mg substituting for Ca in calcite was determined by means of the energy-dispersive spectrometry all of the suspended material. Percentage of Mg substituting for Ca in calcite was determined by means of the energy-dispersive spectrometry

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